THE EFFECTS OF SOIL AMENDMENTS ON TREE GROWTH, YIELD, AND SOIL PROPERTIES IN MATURE MACADAMIA INTEGRIFOLIA ORCHARDS

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

TROPICAL PLANT AND SOIL SCIENCE

2018

By

Russell C. Galanti

THESIS COMMITTEE

Alyssa Cho, Chairperson
Amjad Ahmad
Theodore Radovich

Keywords: Macadamia, soil amendment, mulch, conservation agriculture, sustainable agriculture, biochar, effective microorganisms

ABSTRACT

Current management practices in macadamia production call for the removal of all tree litter from the orchard floor to facilitate nut pick up during harvesting season, which can be up to 10 months of the year. This and derivative management practices lead to degradation of soil and environmental health and reduced nut production. Farmers in Hawaiʻi have expressed interest in using locally sourced alternative soil amendments. A randomized complete block design was implemented on a ~700 acre macadamia farm in Kapa'au, HI. Two sites were selected, one organically managed (Site 1) and one conventionally managed (Site 2). Four blocks consisting of six treatment plots were identified. Several novel soil amendments, including effective microorganisms (EM1®), biochar, and a management practice called soil profiling were identified and were compared to traditional amendments including macadamia husk mulch and wood chip mulch. Including a control treatment, the total treatment amount was six treatments. The effects of these amendments on root growth, SPAD readings, yield/quality in macadamia, and soil carbon (C), nitrogen (N) , pH, and EC were studied. The use of the Minolta SPAD-502 chlorophyll meter to estimate tissue N was evaluated on two macadamia cultivars (HAES 508 'Kakea', and HAES 344 'Keaau), and four sampling times (June 2017, December 2018, and February 2018). SPAD readings had a positive monotonic relationship to leaf tissue N concentrations. For cultivar HAES 508, the February 2018 sampling period had an r^2 value of 0.74. HAES 344 had the highest r^2 (0.24) at the December 2017 sampling period. The Minolta-502 chlorophyll meter can be used for general estimation of tissue N but additional methods need to be considered to refine procedures for direct estimation of N using the chlorophyll meter. Soil profiling resulted in higher yields than any other treatment at a mean of 86.6 kg/tree wet-in-husk. Mean SPAD value was increased by the husk+EM1 and soil profiling treatments from

pretreatment values. Husk+EM1 caused an increase in total root biomass over the study period due to an increase in proteoid root biomass and proportion of proteoid root biomass to total root biomass. The soil profiling treatment was the second lowest in estimated cost per acre to apply and was the highest in estimated partial profit per acre. Soil profiling is a destructive management practice and should be used judiciously until its long-term effects on orchard health are studied. The inoculation of EM1 may have been responsible for the proliferation of proteoid roots under the Husk+EM1 treatment due to microbial inoculation or simple sugar signaling of proteoid root growth. Husk treatments resulted in the greatest increases in NO_3- concentrations and the least decreases in NH_4^+ concentrations. pH was increased at site 2 for the husk, husk+biochar, and soil profiling treatments. EC was increased by the husk+biochar treatment at site 1 by 0.42 mS/cm and all three husk treatments increased soil EC at site 2. Soil C was increased by husk and husk+EM1treatments at site 1. Husk treatments have the potential to increase NO_3- while not causing a significant reduction in NH_4^+ as well as increase soil C. Nitrate is readily leachable from the soil profile, and also requires that the plant expend energy once absorbed. Potential issues with husk mulch use, particularly in combination with biochar or EM1 are increases in pH from 7.7%-10.3% and EC from 66%-100% . While these were statistically significant increases, these increases are not high enough to affect production or plant health after one application. Long term effects of repeated application may cause yield reduction. Mulches generally have more influential effects long-term and results of their effects over a longer period of time would be valuable.

ACKNOWLEDGMENTS

I would like to thank Javier Mollinedo, Colin Hart, Casey Bohan, and Shaun Gutierrez for helping me with data collection. I would like to thank my committee chair, Alyssa Cho, for her exhaustive efforts in advising, funding, and aiding me through my graduate experience, as well as Amjad Ahmad for selflessly helping me through my data collection process. I would like to thank Theodore Radovich for his excellent advice in preparing and executing my research. I would like to thank the University of Hawaiʻi system for the support services and education, as well as the staff at Komohana Research and Extension Center.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

C	Carbon. A chemical element and indicator for soil organic matter.
EC	Electrical conductivity. In the context of this thesis it is a measurement of soil properties, mainly soluble salts.
LAB	Lactic acid bacteria.
EM	Effective microorganisms, a concept used in Korean natural farming.
EM1®	Name for the effective microorganism product consisting of *Lactobacillus casei* that was used in this experiment.
LOI	Loss on ignition. A laboratory method for measuring C.
N	Nitrogen. A chemical element and plant macronutrient in the context of this thesis.
N_2O	Nitrous oxide, a greenhouse gas.
NH_4^+	Ammonium, a plant available form of N.
nm	Nanometer, in the context of this thesis used to measure wavelengths of light
NO_3^-	Nitrate, a plant available form of N.
P	Phosphorous. A chemical element and plant macronutrient in the context of this thesis.
pH	Potential hydrogen. A measure of acidity or basicity in a soil solution through the measurement of hydrogen ions in the solution.
ppb	Parts per billion. A quantity-per-quantity measurement. One part per one billion parts.
SOC	Soil organic carbon.
SOM	Soil organic matter.
TOC	Total organic carbon

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Hawai‘i’s macadamia (*Macadamia integrifolia*, Maiden & Betche) nut industry is the largest macadamia production in the U.S. (USDA, 2017). Despite the importance of macadamia nut production in Hawai‘i there are many challenges that growers continue to face; one being the ability to manage the nutrient status and soil quality of aging orchards. With continual production on orchards that are upwards of 40 years old, the constant removal of organic matter from the orchard floor to facilitate mechanical and hand harvesting has contributed to soil erosion, exposure of roots, and loss of organic matter and nutrients. These effects can result in reduced tree vigor and loss of production. These effects also contribute to environmental degradation through soil quality loss that leads to poor ecosystem functioning (Pimentel et al. 1995). The energy costs related to production of fertilizers and the environmental pollution related to excess fertilizer inputs are causes of environmental issues. For example, the rate at which nitrogen (N) enters the biosphere has increased with the increase in human population (EPA, 2016). There is a strong consensus that excess fertilizer runoff leads to nutrient pollution and promotes eutrophication and harmful algal blooms downstream (Heisler et al., 2008). One part of the solution to these problems is the use of on farm wastes that are converted into mulches and composts. These can improve soil organic matter (SOM) and soil physical properties, which may subsequently prevent soil erosion, improve soil health, reduce the use of manufactured fertilizers, and provide an ecosystem-safe source of nutrients.

Using organic soil amendments is one tenet of sustainable and conservation agriculture. Reducing the reliance on imported fertilizers is a major part of the sustainability of agriculture in Hawaiʻi and it is very important to its stakeholders, based on a 2009 survey by Radovich et al. One potential source of local organic soil amendments could be on-farm byproducts such as mulches and composts made from plant matter. Mulch can increase the yield efficacy of N fertilizers (Gao et al., 2009), improve water use efficiency, reduce erosion, and increase soil organic carbon (SOC) and microbial activity (Cox et al., 2004). The use of mulch in macadamia orchards has the potential to alleviate some of the issues related to soil management that macadamia orchards face, primarily the removal of organic material from the orchards floor due to harvest.

The purpose of this thesis is to provide an evaluation of several soil amendment options for macadamia orchards. This thesis focuses on expanding knowledge of newly available mulch options and tilts the lens on soil and plant health indicators compared to previous related studies. The objectives of this study were to assess the effects of local sources of soil amendments in mature macadamia orchards. The first objective was to determine if and how these soil amendments affect the yield and quality, leaf SPAD values, and root development of macadamia. A partial economic analysis was performed to determine the cost differences and yield benefits between the different treatments. The second objective was to determine if and how the soil amendments affect soil organic matter, plant available nitrogen, pH, and electrical conductivity (EC) in the soil. Additionally, the potential use of the Minolta-502 chlorophyll meter for N determination was assessed by comparing SPAD values to N tissue analyses. The hypotheses for each objective is as follows. For objective one, the treatments are expected to increase yield and quality, root growth, as well as SPAD values over the control treatment. The treatments will cost more than the control, but the yield benefits are expected offset these costs. For objective two, the soil amendment treatments are expected to increase soil carbon (C), N, pH, and EC. Finally, the chlorophyll meter is expected to be relatively accurate in estimating N concentrations in macadamia. The duration of this experiment was one year, from treatment application in March 2017 to March 2018. Baseline soil data was collected in August of 2016.

1.2 Literature review

1.2.1 Botany

Macadamia (*Macadamia integrifolia*) is considered one of the finest gourmet nuts in the world (Nagao, 1992; Stephenson, 2005). The *Macadamia* genus, of the family Proteaceae, is comprised of five currently accepted species, four of which are indigenous to the Australian subcontinent, typically found on the edges of rainforests in southeast Queensland and northeast New South Wales (Stephenson, 2005). These plants evolved physical characteristics indicating adaptation to harsh environments, including sclerophyllous leaves and proteoid roots.

Proteoid roots are a feature found in members of the Proteaceae family and are described as "dense clusters of rootlets of limited growth" by Purnell (1960). Clusters of rootlets develop from the cortex of a proteoid root, with the meristem forming in the pericycle. The normal lifespan of a proteoid root in *M. integrifolia* is two to three months (Malcolm and Trochoulias, 1979). Nutrient deficiencies are implicated in the formation of proteoid roots. Specifically, high P concentrations are linked to decrease in proteoid roots production in Lupin (*Lupinus albus*) with an increase at lower concentrations (Keerthisinghe,1998). Proteoid roots are thought to increase nutrient uptake through the mobilization of immobile nutrients. The increase of root surface area and the exudation of carboxylate organic anions, acid phosphates, phenolics, mucilages, and water facilitate this process (Watt and Evans, 1999).

The physiology of economic interest is the fruit, a dehiscent follicle comprised of the "husk", the "shell", and innermost, the embryo and cotyledons, called the "kernel" with the kernel is the final edible product, and improvement of quality and yield is of greatest interest in the macadamia industry (Nagao, 1992).

1.2.2 Importance of quality

Quality of the kernel determines the payout to producers. Quality standards are outlined by the Hawai'i Department of Agriculture, Quality Assurance Division (1986). Shelled kernels must meet the No. 1 grade requirement if exported, and percent whole kernel at No 1. Grade offers the highest payout. Kernels are qualitatively graded based on being "well developed, "clean", and "dry". These are defined as plump and not shriveled or excessively soft, free from foreign material, and free from surface moisture. Kernels are further graded based on color, wholeness, oil content and defects. Industry standard for a high-quality kernel is 72% to 78% oil content and 1.5% moisture. One way to test this is a float test, in which nuts at or above 72% oil content will float. A nut that sinks is considered immature or defective and not No. 1 grade. Coloration and defects are also assessed during the processing of nuts. Discolored nuts indicate several defects including rancidity, mold and decay. Insect damage is another major issue in the Hawai'i macadamia industry. A number of pests cause defects in the kernel. Tolerances for grade No. 1 are 5% total defects, 1% total mold/dirt/decay/damage, 0.5% soil or extraneous material, 0.1% off-odor/off-color, 0.1% insect infestation, and 0.1% foreign matter. Beyond these quality standards kernels are also graded on "style", as specified by the wholeness of the kernel. There are six styles; Style I 90% whole kernels, Style II 50% wholes and halves, Style III 90% half, Style IV 50% halves and pieces, Style V diced, Style VI chips, Style VII bits and diced, and Style VIII fines. These styles are determined by percentage of kernel pieces that pass through a certain sized opening. Yields are measured by wet in husk weight, wet in shell weight, dry in shell weight and kernel rate. Yield is commonly expressed as nut in shell weight at ~10% moisture content. Quality as reported in this thesis is based on kernel recovery rate of No. 1 grade kernels. Kernel recovery rate is an important indicator because it ultimately defines how much of the total yield is actually usable product. Farmers in the Kohala region average their kernel recovery rate to be around 26-33% when asked at a grower's meeting (Pers. Comm., October 5, 2017).

1.2.3 Related research

Previous research has addressed some aspects of current management practices in macadamia orchards and their effects on tree and soil health. Porter et. al. (2005) noted decreases in proteoid root growth due to this practice of clearing orchard floors of surface organic matter. Research in Australia investigated the negative effects of harvesting equipment (Dalby et al., 2010), finding that using this equipment significantly increases erosion, and along with another study suggested that harvesting practices significantly reduce soil organic matter and nutrients (Reid, 2002). While the use of composts and mulches generally result in prominent long-term effects, some result in short term effects. Mulching increased root growth, available N, and chlorophyll a content in tea olive (*Osmnathus fragrans*) within a year time frame (Xue et al., 2016). Youkhana and Idol (2009) observed an increase in soil C and N within a two-year time frame in a mulched coffee agroecosystem.

1.3 Soil Amendments

1.3.1 Husk mulch

Husk mulch is the most well studied soil amendment in macadamia orchard agroecosystems. Porter et al. (2005) observed increased root growth in plots treated with husk mulch compared to non-mulched plots. This same study also noted increased foliar P, and increased yield in mulched plots. Studies indicate that applying composted macadamia husk in acidic orchard soils increase microbial activity, water holding capacity, pH, soil C and N (Cox et. al., 2004). A cost benefit analysis was performed on macadamia fertilization in Hawaiʻi finding that husk mulch is more expensive to apply versus conventional fertilizers (Bittenbender et al., 1998).

1.3.2 Wood chip mulch

Wood chip mulch can often have a negative effect on nutrient status of soils in the first years of application, although results are often varied (Hoagland et al., 2008; Larsson et al., 1997). Due to the low C:N ratio, wood chip mulch application normally results in a short term decrease in available N due to biological immobilization (Tian et al., 1992). Sinkevičienė et al. (2009) observed a higher amount of phosphorous and a lower yield in sawdust mulch compared to other organic mulches and a control in a three-year study with rotated crops. However, Xue (2016) found that wood chip mulch increased SOM and N over a control in *Osmanthus fragrans* orchards.

1.3.3 Biochar

Biochar is a C rich product created through pyrolysis, the thermal decomposition of biomass at high temperatures. Biochar is mainly under investigation for possible C sequestration in soils for climate change mitigation. Biochar can also improve soil fertility by adding nutrients directly, and by retaining nutrients in the soil and from other sources (Ding et al., 2016; Lehmann and Joseph, 2009). Biochar is linked to increases in cation-exchange-capacity, increased water retention, and increased microbial populations (Ding et al., 2016). However, biochar quality may vary with the production temperature, and not so much with its stock materials (Preston and Schmidt, 2006; McBeath, et al., 2014). Mixed results have been found in yield studies using biochar as a soil amendment. Jeffery et al. (2011) found, through use of meta-analyses of 51 studies, that biochar can have highly variable effects on plant yield in subtropical conditions. In comparison, Eyles et al. (2014) found that biochar did not have an effect on yield in a young apple orchard, but qualified that results may be delayed for several growing seasons and may be expressed where nutrients or water are limited, as was the case in Bartoni et al. (2014).

1.3.4 Effective microorganisms

Effective microorganisms (EM) is a product line developed by Teruo Higa, University of Ryukus, Japan (Higa and Parr, 1994). EM consists of specific mixed cultures of beneficial microorganisms. The constituents in these formulations consist of lactic acid bacteria, yeasts, photosynthetic bacteria, and actinomycetes, along with an array of other organisms. Of particular interest to orchard management would be a potential increase in decomposition rates as a result of an increase in these beneficial microorganisms. Faster decomposition of pruned woody material, husk and shells on site would increase the amount of available organic matter to orchards. Studies evaluating the effectiveness of EM produced mixed results. Jusoh et al. (2013) found accelerated composting rates of rice straw using EM as well as an increase in nutrient elements required for plant growth. Olle and Williams (2013) found that EM increased the yield of vegetables and soil nutrient concentrations.

1.3.5 Soil profiling

Soil profiling, also called fraze mowing, is a cultural practice mainly used in turfgrass management and is a novel technique in macadamia orchards. Soil profiling involves removing the surface layer of soil (0.5 cm to 5 cm) using a rotary spindle with specialized blades. This soil is displaced by force onto the surrounding nearby soil surface. In macadamia, which have copious surface roots, this is essentially root pruning. The soil that is removed is displaced by the force of the soil profiler. In the context of this research the soil profiler is removing soil from the interrows and displacing the soil into the rows directly under the canopies of the trees. There is no available research on the effects of soil profiling on macadamia orchard health specifically, however the effect of root pruning on yield has been studied. Yang et al., 2010 reported that root pruning had no effect on yield of Jujube (*Ziziphus jujube*), but did reduce yield in apple (Feree, 1992) and fruit size in apricots (Arzani et al., 2000).

1.4 SPAD

SPAD is an acronym for soil plant analysis development. The SPAD-502 chlorophyll meter measures the transmittance of two light beams, red (650 nm) and infrared (940 nm), upon initial calibration (Minolta Co. Ltd., No Date). The microprocessor converts these currents into a voltage and stores that value. Next the leaf is measured, the values for the transmission of red and infrared are recorded. The microprocessor outputs a value based on the ratio of the wavelengths transmitted through the leaf relative to the calibrated wavelengths stored in its memory. The equation has been reported differently in several journals (Cerovic et al., 2012; Markwell et al., 1995; Uddling et al., 2007), the most complete equation is given by Naus et al. (2010). The reading output is in an arbitrary SPAD unit that is proportional to the amount of chlorophyll present in the leaf. Chlorophyll is a pigment found in the chloroplasts of plants and is vital for photosynthesis. Chlorophyll content in leaves is often correlated with N status in crops (Evans, 1989; Loomis, 1997). The chlorophyll meter was originally designed for utilization in herbaceous agricultural crops, but studies have indicated that significant correlation can be determined in woody crops (Loh et al., 2002). Leaf nitrogen has been implicated as a successful indicator of mulching effects within a year time span (Smith et al., 2000) and SPAD is an excellent tool for rapidly assessing a large quantity of samples *in situ*. No known research has been conducted on the correlation of SPAD values to leaf tissue N in macadamia.

1.5 Soil properties

1.5.1 Nitrogen

N is one of the most important macronutrients required for plant growth. It is often the most limiting element in regard to plant growth. N is an essential component of amino acids, proteins, enzymes, nucleic acids, and chlorophyll (Taiz and Zeiger, 1998). These functions make nitrogen one of the most important elements in plant growth and subsequently yield and quality of fruit, or in the specific case of macadamia, kernel (Stephenson and Mayer, 1986). It is required in relatively high amounts and is easily lost from agricultural systems. Previous references (Youkhana and Idol, 2009; Xue, 2016) indicated that mulch has varying effects on N status in the soil. Orchard soil N can be increased with the use of mulches even in some cases is competitive with conventional fertilization (Sanchez et al., 2003). One reason a mulch can have a significant effect on soil N is the promotion of microbial biomass that can mineralize and immobilize nutrients.

1.5.2 Soil organic matter

SOM is one of the most important focuses in sustainable agriculture. It acts as a source of inorganic nutrients, microbial food, ion exchange surface, chelation, and is a factor in soil aggregation, root development, water use, disease control, and phytotoxicity (Allison, 1973). Soil C is an indicator for SOM (Awale et al., 2017). The rate of change of soil C is determined by decomposition rates and C inputs; decomposition rates are dependent on climactic and edaphic factors (Paul et al., 1996). Sanchez et. al. (2003) observed a significant increase in orchard soil C in mulch studies compared to conventional fertilization, a similar result was confirmed by other studies (Porter et. al., 2005; Youkhana and Idol, 2009; Xue, 2016). The amount of SOM is estimated by measuring total organic carbon (TOC) which is often measured by loss on ignition (LOI) method. Due to the presence of hydrated minerals on Hawaiʻi (Pers. Comm., Bruce Matthews, January 17, 2017) it is recommended that combustion analysis is used as well for testing accuracy. There is currently no recorded range for an optimal SOC range for macadamia production.

1.5.3 pH and EC

The pH and EC of soils can significantly affect plant growth through control of nutrient availability, microorganism management, and water use (Taiz and Zeiger, 1998). Conventional fertilizers often result in an increased EC and decreased pH (Bunemann et al., 2006). Mulching also has the effect of increasing EC (Hueso-Gonzalez et al., 2014) and can have varying effects on pH depending on the soil type, pH levels, and mulch type. Macadamia require a pH between the range of 5.0-6.5 and macadamia seedlings had severely reduced growth at an EC of 6.6 dS/m (Nagao and Hirae, 1992).

1.6 Summary

Improving macadamia orchard soil quality and providing local on-island sources of agricultural inputs should be a priority in developing sustainable agricultural practices for long term production of macadamia in the state. Previous research has provided a background and justification for the continued study of using soil amendments as potential solutions to plant health and soil degradation. This thesis is divided into three primary chapters. The first is focused on the use of the Minolta-502 chlorophyll meter for determining nitrogen concentrations in macadamia leaf tissue. The second is focused on the effects of soil amendments on macadamia yield, quality of yield, root growth and SPAD readings. The third is on the effects of soil amendments on soil C, N, pH, and EC. This literature adds to the understanding of how local organic inputs can be used in macadamia orchards.

1.7 References

Allison, F.E. (1973). *Soil Organic Matter and its Role in Crop Production*. Amsterdam. Elsevier

Arzani, K., Wood, D., Lawes, G. (2000). Influence of first season application of paclobutrazol, root-pruning and regulated deficit irrigation on second season flowering and fruiting of mature 'Sundrop' apricot trees. *Acta Horticulturae*. 516, 75-82.

Awale, R., Emeson, M.A., Machado, S. (2017). Soil organic carbon pools as early indicators for soil organic matter stock changes under different tillage practices in inland Pacific Northwest. Frontiers in Ecology and Evolution. https://doi.org/10.3389/fevo.2017.00096.

Baronti, S., Vaccari, F.P., Miglietta, F., Calzolari, C., Lugato, E., Orlandini, S., Pini, R., Zulian, C., Genesio, L. (2014). Impact of biochar application on plant water relations in *Vitis vinifera* (L.). *European Journal of Agronomy*. 53, 38–44.

Bittenbender, H. C., Hue, N. V., Fleming, K., Brown, H. (1998). Sustainability of organic fertilization of macadamia with macadamia husk-manure compost. *Communications in Soil Science and Plant Analysis*, *29*(3-4), 409–419.

Bunemann, E., Scwenke, G., Zwieten, L.V. (2006). Impact of agricultural inputs on soil organisms- a review. *Aust. J. Soil. Res.* 44, 379-406.

Cerovic Z.G., Masdoumier G., Ben Ghozlen N., Latouche G. (2012). A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. *Physiologia Plantarum*. 146, 251–260.

Cox, J., Van-zwieten, L., Ayres, M., Morris, S. (2004). Macadamia husk compost improves soil health in sub-tropical horticulture. *Super Soil: 2004 3rd Australian New Zealand Soils Conference*. University of Sydney, Australia.

Dalby, T., Cox, J., Morris, S. (2010). Harvest equipment and soil erosion in a macadamia orchard. 19th World Congress of Soil Science, Soil Solutions for a Changing World, 1-6 August 2010, Brisbane, Australia.

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., Zeng, G., Zhou, L. Zheng, B. (2016). Biochar to improve soil fertility. A review. *Agronomy Sustainable Development.* 46, 1-18. DOI 10.1007/s13593-016-0372-z

Environmental Protection Agency. (2016). *Climate Change Indicators in the United States: Atmospheric Concentrations of Greenhouse Gases*. Accessed January 11, 2018. https://www.epa.gov/sites/production/files/2016-08/documents/print_ghg-concentrations-2016.pdf

Evans, J. (1989). Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia*. 78(*1*), 9-19.

Eyles, A., Corkrey, R., Hardie, M. (2015). Impact of biochar amendment on the growth, physiology and fruit of a young commercial apple orchard. *Trees. 29*(6), 1817-1826.

Feree, D. (1992). Time of root pruning influences vegetative growth, fruit, size, biennial bearing, and yield of 'Jonathan' apple. *Journal of the American Society of Horticulture Science.* 117(*2*), 198-202.

Gao, Y., Li, Y., Zhang, J., Liu, W., Dang, Z., Cao, W., Qiang, Q. (2009). Effects of mulch, N fertilizer, and plant density on wheat yield, wheat nitrogen uptake, and residual soil nitrate in a dryland area of China. *Nutrient Cycling in Agroecosystems.*

Giambelluca, T.W., X. Shuai, M.L. Barnes, R.J. Alliss, R.J. Longman, T. Miura, Q. Chen, A.G. Frazier, R.G. Mudd, L. Cuo, and A.D. Businger. (2014). Evapotranspiration of Hawaiʻi. Final report submitted to the U.S. Army Corps of Engineers—Honolulu District, and the Commission on Water Resource Management, State of Hawaiʻi.

Hawaiʻi Department of Agriculture, Quality Assurance Division. (1986). *Standards for* Hawaiʻi *grown shelled macadamia nuts.* Accessed January 16, 2018. http://hdoa.hawaii.gov/qad/files/2012/12/AR-41-47.pdf

Heisler, J., Glibert, P., Burkholder, J., Anderson, D., Cochlan, W., Dennison, W., Gobler, C., Dortch, Q., Heil, C., Humphries, E., Lewitus, A., Magnien, R., Marshall, H., Sellner, K., Stockwell, D., Stoecker, D., Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific concensus. *Harmful Algae*. *8*(1), 3-13.

Higa, T, Parr, J. (1994). *Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment*. International Nature Farming Research Center, Atami, Japan.

Hoagland, L., Carpenter-Boggs, L., Granatstein, D., Mazzola, M., Smith, J., Peryea, F., Reganold, J.P. (2008). Orchard floor management effects on nitrogen fertility and soil biological activity in a newly established organic apple orchard. *Biology and Fertility of Soil*s. 45, 11-18.

Hueso-Gonzalez, P., Martinez-Murillo, J.F., Ruiz-Sinoga, J.D. (2014). The impact of organic amendments on forest soil properties under Mediterranean climatic conditions. *Land degradation & Development.* 25, 604-612.

Jeffery S, Verheijen FFA, van der Velde M, Bastos AC. (2011). A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agriculture, Ecosystems & Environment*. 144,175–187.

Jusoh, M., Manaf, L., Latiff, P. (2013). Composting of rice straw with effective microorganisms (EM) and its influence on compost quality. *Irnanian Journal of Environmental Health Sciences & Engineering*. *10*(17).

Keerthisinghe G, Hocking PJ, Ryan PR, Delhaize E. (1998). Effect of phosphorus supply on the formation and function of proteoid roots of white lupin (Lupinus albus L.). *Plant Cell Environ* 21, 467–478.

Larsson L., Stenberg B., Torstensson L. (1997). Effects of mulching and cover cropping on soil microbial parameters in the organic growing of black currant. *Communications in Soil Science and Plant Analysis*. 28, 913-925.

Lehmann, J., Joseph, S. (2009). *Biochar for Environmental Management : Science and Technology*. Sterling, VA. Earthscan

Loh, F., Grabosky, J., Bassuk, N. (2002). Using the SPAD 502 meter to assess chlorophyll and nitrogen content of benjamin fig and cottonwood leaves. *HortTechnology*. *12*(4), 682-686.

Loomis, R. (1997). On the utility of nitrogen in leaves. *Proceedings of the National Academy of Science.* 94, 13378-13379.

Malcolm, H., Trochoulias, T. (1992). Proteoid roots help macadamia nut trees. *Agriculture Gazzette of New South Wales*. *90*(1), 42-43.

Markwell J., Osterman J.C., Mitchell J.L. (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynthesis Research*. 46, 467–472.

McBeath, A.V., Smernik, R.J., Krull, E.S., Lehmann, J. (2014). The influence of feedstock and production temperature on biochar carbon chemistry: A solid-state ^{13}C NMR study. Biomass and Bioenergy (60): 121-129.

Minolta Co., Ltd. (No Date). Chlorophyll Meter SPAD-502. Retrieved from http://www.johnmorris.com.au/files/product/attachments/18012/160254_manual_instr.pdf

Nagao, M., Hirae, H. (1992). Macadamia: Cultivation and Physiology. *Critical Reviews in Plant Science*, *10*(5), 441-470.

Naus J., Prokopova J., Rebicek J., Spundova M. (2010). SPAD chlorophyll meter reading can be pronouncedly affected by chloroplast movement. *Photosynthesis Research*. 105, 265–271.

Olle M., Williams I. (2013). Effective microorganisms and their influence on vegetable production – a review. *Journal of Horticultural Science & Biotechnology*. *88*(4), 380 – 386.

Paul, E., Paustian, K., Elliot, E., Cole, V. (1996). *Soil Organic Matter in Temperate Agroecosystems: Long Term Experiments in North America.* Boca Raton, FL. CRC Press

Porter, G., Yost, R., Nagao, M. (2005). the Application of Macadamia Nut Husk and Shell Mulch To Mature Macadamia Integrifolia To Improve Yields, Increase Nutrient Utilization, and Reduce Soil P Levels. *Western Nutrient Management Conference*. *6*, 226–233. Salt Lake City, UT.

Preston C.M., and Schmidt M.W.I. (2006). Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. Biogeosciences 3: 397-420.

Purnell HM. (1960). Studies of the family Proteaceae. I. Anatomy and morphology of the roots of some Victorian species. *Aust J Bot* 8, 38–50.

Radovich, T. J. K., Cox, L. J., Hollyer, J. R. (2009). Overview of Organic Food Crop Systems in Hawaiʻi. College of Tropical Agriculture and Human Resources. SA-3.

Reid, G. .(2002). Soil and nutrient loss in macadamia lands: a pilot study. Horticulture Australia Report MC 98011.

Sanchez, J., Edson, C., Bird, G., Whalon, M., Wilson, T., Harwood, R., Kizilkaya, K., Nugent, J., Klein, W., Middleton, A., Loudon, T., Mutch, D., Scrimger, J. (2003). Orchard floor and nitrogen management influences soil and water quality and tart cherry yields. *Journal Amer. Soc. Hort. Sci. 128*(2), 277-284.

Sinkevičienė, A., Jodaugienė, D., Pupalienė R., Urbonienė, M. (2009). The influence of organic mulches on soil properties and crop yield. *Agronomy Research. 7*(1) 485-491.

Smith, M., Carroll, B., Cheary, B. (2000). Mulch Improves Pecan Tree Growth during Orchard Establishment. *HortScience. 35*(2), 192-195.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov/. Accessed [October/25/2016].

Stephenson, R., Cull, B., Mayer, D. (1986). Effects of site, climate, cultivar, flushing, and soil and leaf nutrient status on yields of macadamia in south east Queensland. *Scientia Horticulturae. 30*(3), 227-235

Stephenson, R. (2005). Macadamia: Domestication and Commercialisation. *Chronica Horticulturae*. ISSN 0578-039X. V.45.2. June 2005. International Society for Horticultural Science.

Taiz, L., Zeiger, E. (1998). *Plant Physiology.* Sunderland, MA. Sinauer Associates, Inc.

Tian, G., Kang, B., Brussard, L. (1992). Biological effects of plant residues with contrasting chemical compositions under humid tropical conditions-Decomposition and nutrient release. *Soil Biology and Biochemistry*. *24*(10), 1051-1060.

Uddling J., Gelang-Alfredsson J., Piikki K., Pleijel H. (2007). Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynthesis Research*. 91, 37–46.

United States Department of Agriculture. (2017). *Noncitrus Fruits and Nuts 2016 Summary.* ISSN: 1948-2698. USDA, National Agricultural Statistics Service.

Yang, S., Xing, S., Liu, C., Du, Z., Wang, H., Xu, Y. (2010). Effects of root pruning on the vegetative growth and fruit quality of Zhanhuadongzao trees. *Horticulture Science* (Praque). 37, 14-21.

Youkhana, A., Idol, T. (2009). Tree pruning mulch increases soil C and N in a shaded coffee agroecosystem in Hawaiʻi. *Soil Biology & Biochemistry*. 41, 2527-2534.

Xue, N., Weiling, S., Huanchao, Z., Xiulian, Y., Lianggui, W. (2016). Effects of mulching on soil properties and growth of tea olive (*Osmanthus fragrans*). *PLoSONE 11*(8), 1-11.

CHAPTER 2

EVALUATING THE USE OF THE MINOLTA-502 CHLOROPHYLL METER FOR NONDESTRUCTIVE AND RAPID ESTIMATION OF LEAF TISSUE NITROGEN IN MACADAMIA

2.1 Abstract

Nitrogen (N) management in macadamia orchards is an important concern for growers. Leaf tissue analysis is the accepted method for determining N status in macadamia. This process is expensive and time consuming, as receiving test results is dependent on the punctuality of the lab being used. The Minolta SPAD-502 chlorophyll meter has been used in other crops to estimate N status in plants through estimation of the amount of chlorophyll in leaf tissue. The use of the chlorophyll meter in two macadamia cultivars, at two different locations and four different time periods on Hawaiʻi Island was assessed. Leaf samples were collected based on tissue sampling protocol, SPAD values were collected, and leaves were sent for N analysis using a Leco CN-2000 analyzer. Data were analyzed using linear regression. Leaf tissue N concentrations had a positive monotonic relationship to SPAD values for both cultivars, both locations, and all sampling periods. The sampling period of April 2017 for HAES 508 had the greatest r^2 value for the linear regression at 0.85. The February 2018 sampling period had an r^2 value for the linear regression of 0.74. HAES 344 had the highest r^2 value for the linear regression of 0.24 in the December 2017 sampling period. The slopes of the two cultivars for June 2017 were different from each other, suggesting that N recommendations need to be customized for specific macadamia cultivars if sampled in summer. The Minolta-502 chlorophyll meter can be used for general estimation of tissue N but additional methods need to be considered and researched to potentially refine procedures for direct estimation of N when using the chlorophyll meter.

2.2 Introduction

Management of N in macadamia (*Macadamia integrifolia*) orchards is of primary concern for growers. Plant nutrient status is a factor that affects plant vigor, yield, and quality of the macadamia kernel. Managing the N status in these orchards is therefore important. N is lost from the macadamia through the harvesting process. In every 100 kg of harvested wet-in-husk nuts, 405 g of N is removed from the plant and agroecosystem (Nagao and Hirae, 1992). If not returned to the agroecosystem, low N levels will result in poor plant growth (Nagao and Hirae, 1992; Stephenson et al, 2002). Excess N applications can lead to fertilizer runoff and pollution with harmful environmental side effects, as well as financial losses (Heisler et al., 2008). Prescription-based fertilization recommendations can reduce incidences of inappropriate N fertilization applications.

Analysis of leaf tissue for nutrient concentrations is the accepted method to assess the nutritional status of macadamia crops (Cooil et al., 1953; Guest, 1943; Hirae, 1976; Wallace, 1971). Leaf samples are collected in the spring, after harvest and before the vegetative flush. Australian studies on optimal leaf tissue N are very similar with the recommendation from Hawaii at 1.45%-2% (Bittenbender and Hirae, 1990) with recommendations in Australia ranging from 1.4%-1.5% (Stephenson and Cull, 1986) of N based on dry leaf weight. Tree growth is reduced when N leaf concentrations are below 1.22% (Nagao and Hirae, 1992). The current practice of collecting leaf samples is time consuming and expensive for growers. Furthermore, leaf selection criteria for tissue analysis is subjective and timing the collection based on plant ontogeny can make collection even more complicated. Additionally, there are only a few labs available to growers and these are often several hours away or have limited availability. Because of the time and expense only a small subset of trees is selected for analysis, which can lead to inaccurate recommendations for the rest of the orchard. Having an affordable and rapid method to assess N levels *in situ* would be a great benefit to macadamia growers.

Chlorophyll and biomass related to it account for around 75% of leaf N (Loomis, 1997), making chlorophyll a fairly accurate indicator for N status in leaves. The SPAD-502 chlorophyll meter (Konica Minolta, Osaka, Japan) is a portable handheld device developed to quickly and non-destructively measure foliar N. The chlorophyll meter measures relative chlorophyll concentration. The meter emits two wavelengths of light at 650 nm and 940 nm. Chlorophyll absorbs light at 650 nm and reflect light at 940 nm. The amount of each wavelength that is transmitted through the leaf is collected by a photodiode in the meter and converted to volts and subsequently valued. These values are used in an equation to form an empirical relationship by the meter's microprocessor. The equation has been reported differently in several journals (Cerovic et al., 2012; Markwell et al., 1995; Uddling et al., 2007), the most complete equation is given by Naus et al. (2010).

$$\text{SPAD} = k \times \log \frac{\%\ \text{transmittance at 940 nm}}{\%\ \text{transmittance at 650 nm}} + C \qquad \text{Eq. (1)}$$

Where:

k is a slope coefficient; *C* is an offset value

k and *C* in this equation are confidential property of the Minolta Co. Ltd. The reading output (SPAD) is in an arbitrary SPAD unit between 0 and 100 that is proportional to the amount of chlorophyll, and significantly correlated to N present in a leaf using regression analysis.

The chlorophyll meter was originally developed for measuring foliar N in rice (Chubachi et al., 1986). The meter's use has since been expanded to include research in hardwood and fruit trees (Chang and Robinson, 2003; Hardin et al., 2012; Netto et al., 2005). A chlorophyll meter may have use for assessing tissue N levels in macadamia orchards. The objective of this study was to evaluate the relationship between SPAD values obtained *in situ* to correspondent N tissue analysis. Leaf N concentration are expected to have a positive relationship with SPAD values. Macadamia cultivars and sampling times are expected to have different SPAD values and leaf N concentrations.

2.3 Materials and methods

2.3.1 Plant material

The material selected for this study was obtained from plants at two private farms on Hawai‘i Island. All samples were collected as composite samples consisting of 15 leaves per sample from each tree. Samples were initially collected from 8 *M. integrifolia* ‘Kakea’ (HAES 508) trees in Kapa’au, HI on 12 April 2017 as a preliminary sample. Samples were collected from 50 *M. integrifolia* ‘Kakea’ (HAES 508) trees in Kapa’au on 15 June 2017. Samples were collected from 32 *M. integrifolia* ‘Kau’ (HAES 344) trees in Pahala, HI on 13 June 2017. Samples were collected from 16 *M. integrifolia* ‘Kau’ (HAES 344) trees in Pahala, HI on 18 December 2017. The experiment was designed to emulate the standard sampling protocol for tissue sample collection in macadamia.

2.3.2 Sampling protocol and nitrogen analysis

Branches were selected based on their stage of growth; the criteria were that the terminal bud of the branch was not flushing, and that the branch tissue was woody directly up to the terminal bud to ensure uniform maturity. Selected leaves were exposed to full sun, mature, undamaged and on the second node from the terminal bud. Macadamia stem nodes have a whorl arrangement usually consisting of three leaves. All three leaves were selected from the same whorl to minimize destructive sampling. The Minolta SPAD-502 chlorophyll meter was calibrated before use. Each leaf was measured using the chlorophyll meter by placing the measuring head onto the middle of the leaf blade, adjacent to the leaf mid rib, and then recording the SPAD value. The leaf was then collected for composite sampling. Leaves were taken to Komohana Research and Extension Center, rinsed, air dried and sent to University of Hawaiʻi at Mānoa's Agricultural Diagnostic Service Center (Honolulu, HI). Total nitrogen was analyzed at ADSC using a Leco CN-2000 analyzer.

2.3.4 Statistical analysis

All data were subjected to analysis using JMP Pro version 13.1 (SAS Institute Inc., NC, USA). Linear regressions were fitted for foliar N concentrations on SPAD values. One-Way ANCOVA with interaction simple slopes test model was used to determine differences between slopes.

2.4 Results and discussion

For HAES 508, foliar N concentrations ranged from 0.25% to 2.76% in the June 2017 sampling period and from 0.88% to 1.93% in February 2018 sampling period. Mean N concentrations were lower in June (1.15%) compared to February (1.50%) (Fig. 2.1). SPAD readings ranged from 41.7 to 57.5 in June 2017 and from 35.6 to 55.9 in February of 2018 (Fig. 2.2). An inverse relationship in changes in range between N concentration and SPAD readings was observed for the two periods. Equations predicting foliar N from SPAD readings is listed by month/year and variety in Table 2.1. Regression analyses indicated a positive monotonic linear relationship between leaf N concentration to SPAD readings. r^2 values for the linear regression were highest for February 2018 ($r^2 = 0.74$) (Fig. 2.3). This r^2 value for the linear regression was much closer to the preliminary samples collected in April of 2017 ($r^2 = 0.85$) (Fig. 2.4). These r^2 values of 0.74 and 0.85 suggest the chlorophyll meter can predict N tissue concentrations during the late winter to early spring period. Spring is the currently recommended time to sample for leaf tissue N in macadamia, which this study corroborates as an accurate time to measure for N concentration in leaf tissue. The slopes of the linear regressions were compared. Analysis indicated that the slopes for the two sampling periods were different ($p = 0.0126$) (Table 2.2). This analysis between the slopes of the sampling times (Table 2.2) in addition to the low r^2 value for the June 2017 linear regression (Fig. 2.5) indicate that SPAD use to determine tissue N concentrations might not be representative for June sampling periods in Hawai‘i. Seasonal differences in N concentration prediction from SPAD readings were also seen in cottonwood and Benjamin fig (Loh et al., 2002).

For HAES 344, foliar N concentrations ranged from 1.25% to 2.85% for June 2017 and from 0.94% to 1.91% for December 2017. Mean N concentrations were higher in June (1.98%) than December (1.41%) ($p < 0.0001$) (Fig. 2.1). This pattern of decreasing range of N concentration in December compared to June was similar in HAES 508. SPAD readings ranged from 44.5 to 59.1 for June and from 45 to 55.1 for December (Fig. 2.2). This pattern of decreasing range in SPAD values in December compared to June is dissimilar that found in HAES 508. As range lowered for N concentration in December, so did SPAD readings. The relationship between changes in N concentration and SPAD value range suggests a difference in how these two *M. integrifolia* cultivars affect SPAD values. The difference could also be due to other elemental concentrations that affect chlorophyll or environmental differences affecting the trees. The r^2 value for the linear regression for June ($r^2= 0.20$) (Fig. 2.6) was close to the r^2 value (= 0.24) for December (Fig. 2.7). The slopes of the linear regressions were compared (Table 2.2). Analysis indicated that the slopes were not significantly different ($p = 0.7491$). This contradiction to the slope comparison in HAES 508 can be explained by the sampling times.

HAES 508 and HAES 344 were compared for June 2017 sampling time (Table 2.3). Samples were taken at similar periods for this sampling period and could be compared to each other. Both N concentration and SPAD values were higher in HAES 344 than HAES 508 in June 2017. This further corroborates some ability of the chlorophyll meter to predict N concentration. Considering the large differences in N concentration between these two trees during the June 2017 sampling period, generalized optimum leaf tissue N recommendations may need to be refined for popular varieties if sampling during non-recommended sampling times. The winter sampling times had much more similar mean N concentration than the summer sampling times for the two varieties (Fig. 2.1). Different *M. integrifolia* cultivars may have discrete levels of leaf tissue N while having similar or dissimilar health and yield. Potential techniques to improve the accuracy of these equations could be expressing N as mass per leaf area (Wu et al., 1995), and by including leaf moisture content as a variable in the regression (Chang and Robinson, 2002). Further limitations in the use of the chlorophyll meter are variations in leaf thickness and color on a leaf subsample. This can decrease the accuracy of the meter (Chapman and Barreto, 1997). Multiple readings could be taken from each leaf subsample and averaged.

2.5 Conclusion

This experiment indicates that the chlorophyll meter can be used to provide relative estimation of leaf N concentration. The regression with the highest correlation explained 85% of the variation around the mean for correlation of SPAD value and N concentration; the lowest explained only 20%. Using higher order regression polynomials may be a way to improve response. The response of the chlorophyll meter was dependent on season and *M. integriolfia* cultivar, so distinct equations may need to be developed based on cultivar and sampling period. Changes in the chlorophyll meter's response based on seasonality is related to rhythmic vegetative growth patterns (Nagao, 1992), and seasonal source-sink relationships between vegetative and reproductive growth; developing fruit during the summer act as a N sink (Fletcher et al., 2009). Some potential options to improve the chlorophyll meter's response without developing distinct equations for cultivars would be multiple measurements on leaf subsamples, factoring in leaf moisture content, and using N mass per leaf area. This study corroborates previous statements that the chlorophyll meter can be used in comparative studies for general changes in leaf tissue N concentration and is not suitable for absolute estimates (Hardin et al., 2012; Loh et al., 2002).

Further study could focus on using improved methods for improving chlorophyll meter response and including more macadamia cultivars and sampling periods.

2.6 Tables

Table 2.1- Linear regression models predicting foliar N % in two *M. integrifolia* cultivars at four sampling periods from SPAD chlorophyll meter readout values.

Sample period and variety	Model	r^2	p-value
April 2017, HAES 508	N = -11.75024 + 0.2581211*SPAD	0.85	**0.0010** [z]
June 2017, HAES 344	N = -1.115881 + 0.0578416*SPAD	0.20	**0.0116**
June 2017, HAES 508	N = -3.323193 + 0.0874522*SPAD	0.29	**<0.0001**
December 2018, HAES 344	N = -0.98932 + 0.0460859*SPAD	0.24	**0.0488**
February 2018, HAES 508	N = -0.649068 + 0.043715*SPAD	0.74	**<0.0001**

[z] Bold text indicates that the coefficient value is not equal to 0 with a $P \leq 0.05$.

Table 2.2- One-Way ANCOVA testing differences between the slopes of regression models comparing SPAD chlorophyll meter readout values to foliar N % using the student's t-test.

Comparison of slopes	Estimate	*t*-ratio	p-value
June 2017 HAES 508 vs. February 2018 HAES 508	0.0437	2.54	**0.0126** [z]
June 2017 HAES 344 vs. December 2018 HAES 344	0.0117	0.04	0.7491
June 2017 HAES 508 vs. June 2017 HAES 344	0.0296	1.01	0.3155

[z] Bold text indicates a statistically significant difference between slopes with a $P \leq 0.05$.

Table 2.3- One-Way ANOVA testing difference between means of Foliar N % and SPAD values for the two cultivars sampled in June 2017 using the student's t-test.

Date and cultivar	Nitrogen (%)	SPAD
June 2017 HAES 344	1.98	53.4
June 2017 HAES 508	1.15	51.2
Significance	**p = <0.0001**[z]	**p = 0.0006**

[z] Bold text indicates a statistically significant difference with a $P \leq 0.05$.

2.7 Figures

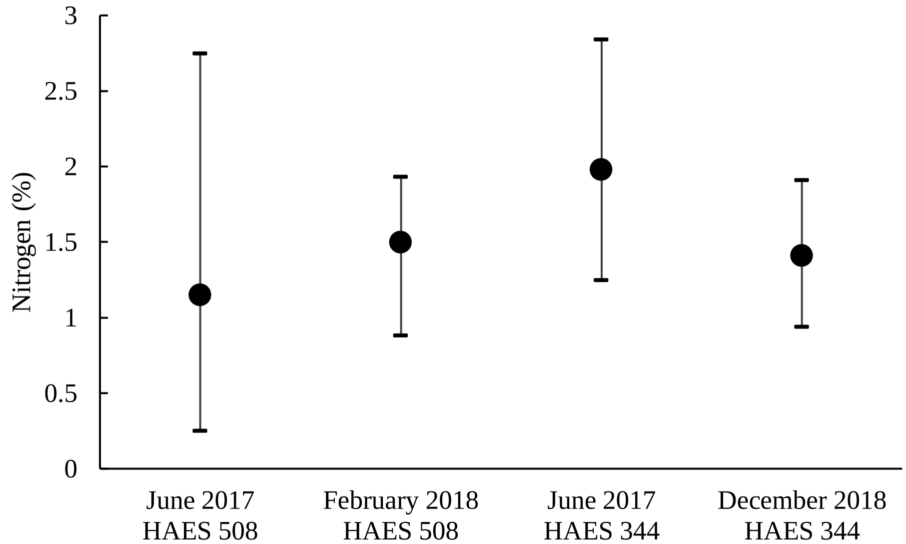

Figure 2.1- Ranges and means for N concentrations of two macadamia cultivars at four different sampling times.

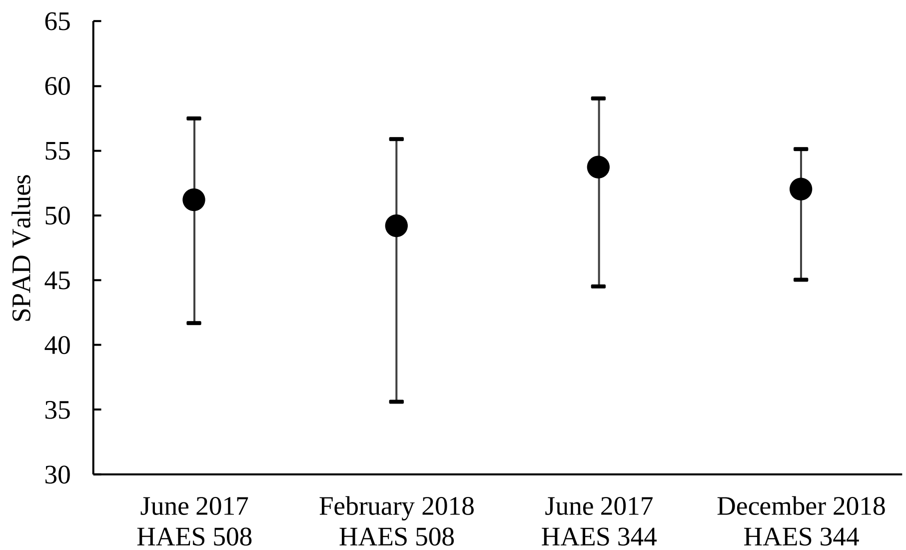

Figure 2.2- Ranges and means for SPAD values of two macadamia cultivars at four different sampling times.

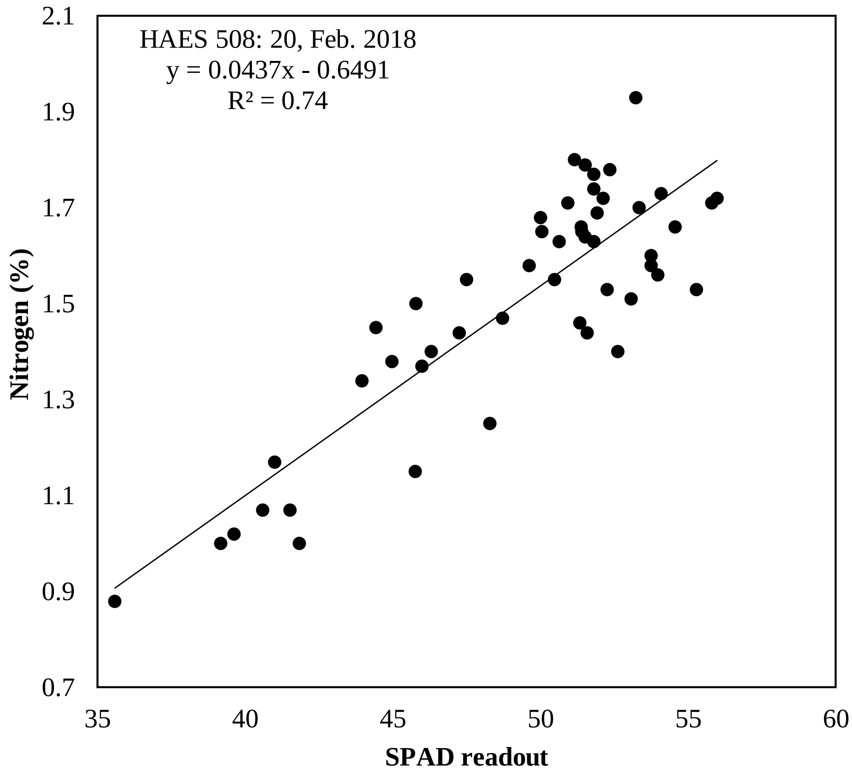

Figure 2.3- Regression analysis for Leaf N (%) by SPAD value for 20 February 2018 *for M. integrifolia* HAES 508.

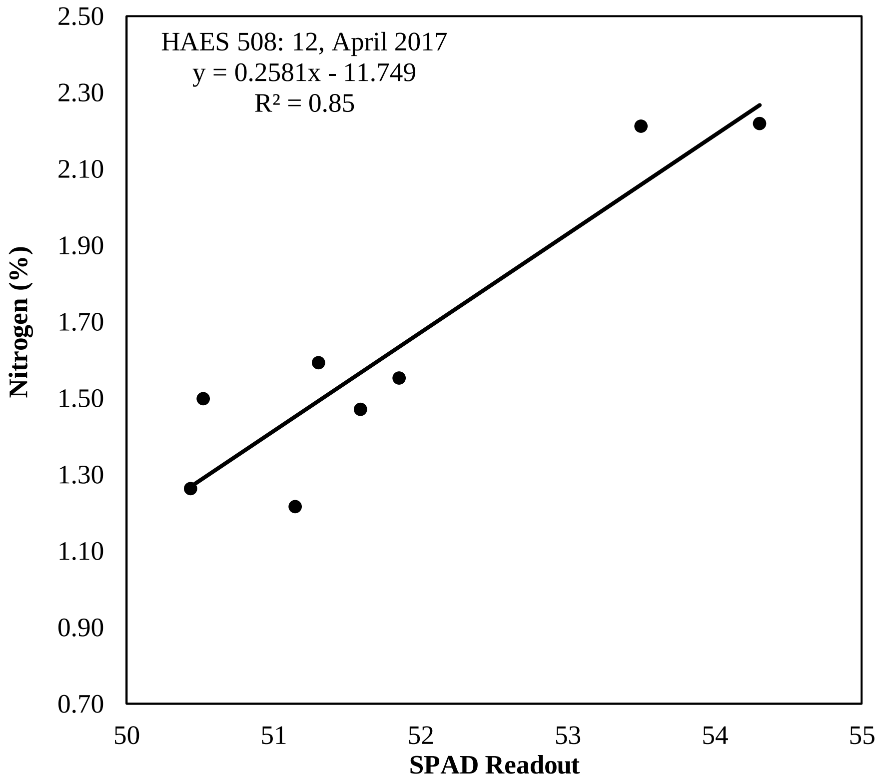

Figure 2.4- Regression analysis for Leaf N (%) by SPAD value for 12 April 2017 for *M. integrifolia* HAES 508.

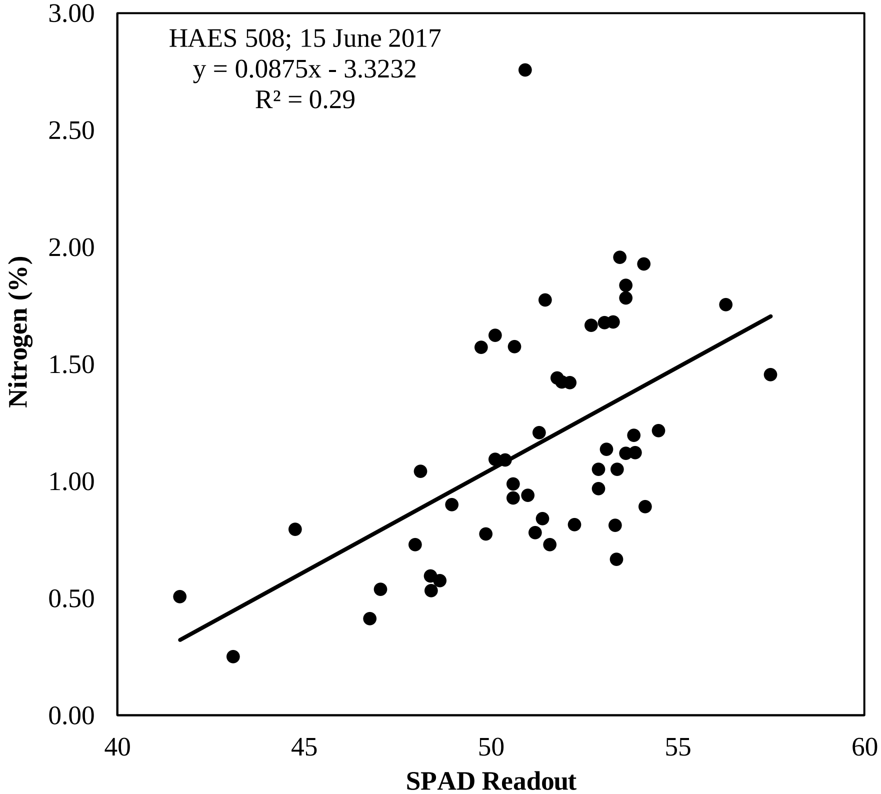

Figure 2.5- Regression analysis for Leaf N (%) by SPAD value for 15 June 2017 for *M. integrifolia* HAES 508.

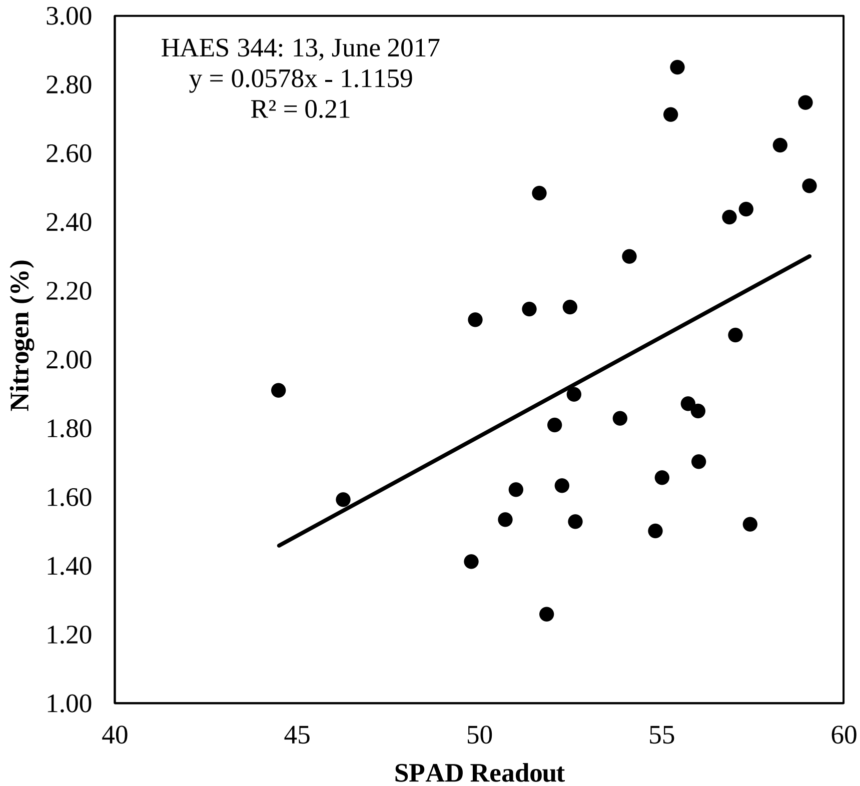

Figure 2.6- Regression analysis for Leaf N (%) by SPAD value for 13 June 2017 for *M. integrifolia* HAES 344.

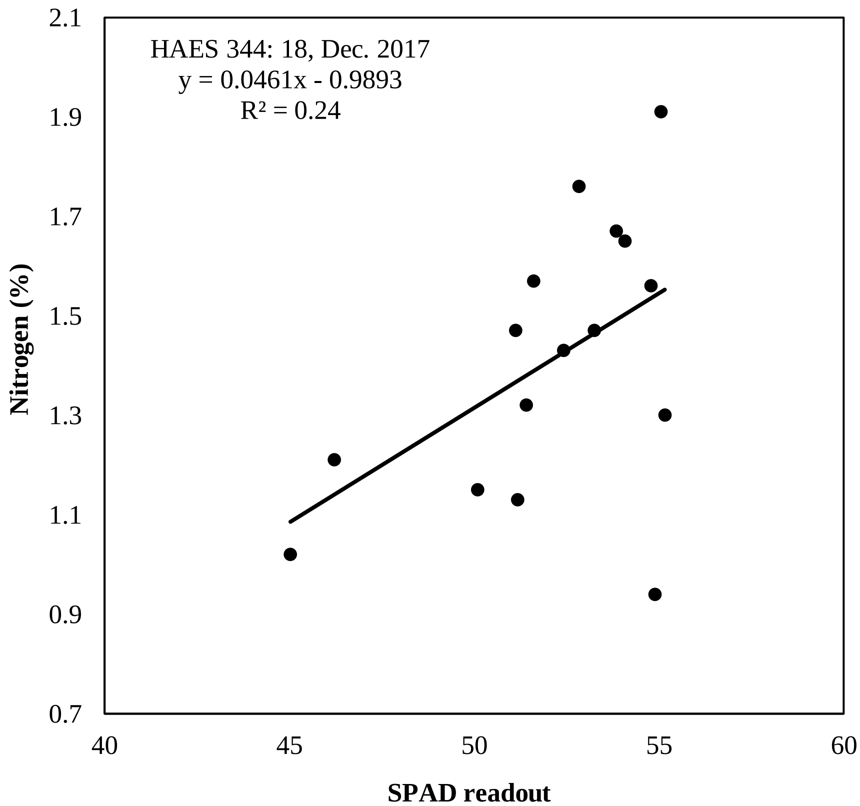

Figure 2.7- Regression analysis for Leaf N (%) by SPAD value for 18 December 2017 for *M. integrifolia* HAES 344.

2.8 References

Bittenbender, H., Hirae, H. (1990). Common problems of macadamia nut in Hawai‘i. *College of Tropical Agriculture and Human Resources University of Hawaii Research Extension Series #112*.

Chang, S., Robinson, D. (2003). nondestructive and rapid estimation of hardwood foliar nitrogen status using the SPAD-503 chlorophyll meter. *Forest Ecology and Management* 181, 331-338.

Chapman, S.C., Barreto, H.J. (1997). Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. *Agronomy Journal.* 89, 557-562.

Cerovic Z.G., Masdoumier G., Ben Ghozlen N., Latouche G. (2012). A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. *Physiologia Plantarum*. 146, 251–260.

Chubachi, T., Asano, I., Oikawa, T. (1986). The diagnosis of nitrogen nutrition of rice plants using chlorophyll meter. *Soil Science and Plant Nutrition.* 57, 190–193.

Cooil, B., Awada, M., Nakata, S., Nakayama, M. (1953). Leaf concentrations associated with deficiencies of nitrogen, potassium, and phosphorous in macadamia. *Hawaii Agricultural Experiment Station University of Hawaii Program Notes No. 88.*

Fletcher, A., Rennenberg, H., Schmidt, S. (2009). Nitrogen partitioning in orchard grown *Macadamia integrifolia. Tree Physiology.* 30, 244-256.

Guest, P. (1953). A comparison of certain chemical constituents of green and chlorotic macadamia leaves. *Proceedings of the American Society of Horticultural Science.* 42, 104.

Hardin, J., Smith, M., Wekler, P., Cheary, B. (2012). In situ measurement of pecan leaf nitrogen concentration using a chlorophyll meter and vis-near infrared multispectral camera. *HortScience. 47*(7), 955-960.

Heisler, J., Glibert, P., Burkholder, J., Anderson, D., Cochlan, W., Dennison, W., Gobler, C., Dortch, Q., Heil, C., Humphries, E., Lewitus, A., Magnien, R., Marshall, H., Sellner, K., Stockwell, D., Stoecker, D., Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific concensus. *Harmful Algae*. *8*(1), 3-13.

Hirae, H. (1976). Leaf and soil analysis in macadamia. *Hawaii Macadamia Producers’ Assoc. 16th Annual Proceedings.* p. 65.

Loh, F., Grabosky, J., Bassuk, N. (2002). Using the SPAD 502 Meter to assess chlorophyll and nitrogen content of Benjamin fig and cottonwood leaves. *HortTechnology*. *12*(4), 682-686.

Loomis, R. (1997). On the utility of nitrogen in leaves. *Proceedings of the National Academy of Science.* 94, 13378-13379.

Markwell J., Osterman J.C., Mitchell J.L. (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynthesis Research*. 46, 467–472.

Nagao, M., Hirae, H. (1992). Macadamia: Cultivation and Physiology. *Critical Reviews in Plant Science*. *10*(5), 441-470.

Naus J., Prokopova J., Rebicek J., Spundova M. (2010). SPAD chlorophyll meter reading can be pronouncedly affected by chloroplast movement. *Photosynthesis Research*. 105, 265–271.

Netto, A.T., Campostrini, E., Oliveira, J.G., Bressan-Smith, R.E. (2005). Photosynthetic pigments, nitrogen, chlorophyll *a* fluorescence and SPAD-502 readings in coffee leaves. *Scientia Horticulturae.* 104, 199-209.

Stephenson, R., Cull, B. (1986). Standard leaf nutrient levels for bearing macadamia trees in South East Queensland. *Scientia Horticulturae.* 30, 73.

Stephenson, R., Cull, B., Mayer, D. (1986). Effects of site, climate, cultivar, flushing, and soil and leaf nutrient status on yields of macadamia in south east Queensland. *Scientia Horticulturae. 30*(3), 227-235.

Uddling J., Gelang-Alfredsson J., Piikki K., Pleijel H. (2007). Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynthesis Research*. 91, 37–46.

Wallace, A. (1971). Mineral composition of macadamia leaves. *Regulation of Micronutrient Status of Plants by Chelating Agents and Other Factors.* Ann Arbor, MI. Edwards Brothers, Inc.

Wu, F.B., Wu, L.H., Xu, F.H. (1998). Chlorophyll meter to predict nitrogen side dress requirements for short-season cotton (Gossypium hirsutum L.). *Field Crop Research.* 56, 309-314.

CHAPTER 3

THE EFFECTS OF SOIL AMENDMENTS ON YIELD AND QUALITY, LEAF SPAD VALUES, AND ROOT GROWTH IN MACADAMIA

3.1 Abstract

Macadamia nut production in Hawai‘i is a $42 million dollar industry and is the largest macadamia nut industry in the U.S. (USDA, 2017). Soil management in aging orchards can have adverse effects on tree health and yield. Several new soil amendment options have been identified and their effects on tree health parameters were assessed. Macadamia husk mulch, husk+biochar, husk+effective microorganisms (EM1®), soil profiling, and wood chip mulch were applied in a randomized complete block experimental design. Yield, yield quality, root growth, and SPAD values were assessed over the year-long study. A partial cost benefit analysis was performed to compare the costs and benefits in terms of yields for each treatment. Soil profiling resulted in higher yields than any other treatment at a mean of 86.6 kilograms wet-in-husk. No treatments significantly affected nut quality or dry kernel weight. Nut quality was affected by harvesting time, with the earliest harvesting (August 2017) period resulting in the highest kernel recovery rate (33%). SPAD value was increased by the husk+EM1 treatment and soil profiling treatments from pre-treatment values, but no treatments caused significant differences in SPAD values at the end of the study. The Husk+EM1 treatment had the greatest effect on root growth. Husk+EM1 caused an increase in total root biomass over the study period due to increases in proteoid root biomass and proportion of proteoid root biomass. The soil profiling treatment was the second lowest in estimated cost per acre to apply and was the highest in estimated profit per acre. Soil profiling is a destructive management practice and should be

used judiciously until its long-term effects on orchard health are studied. The inoculation of EM1 may have been responsible for the proliferation of proteoid roots under this treatment.

3.2 Introduction

Macadamia (*Macadamia integrifolia*, Maiden & Betche) nut production is estimated as a 42.0 million-pound, $42.0 million dollar industry in Hawai‘i (USDA, 2017), the largest macadamia production in the U.S. Current management practices during harvesting call for the removal of all litter-fall from the trees from the orchard floor to facilitate nut pick up. This removes sources of organic matter and may lead to soil degradation (Reid, 2002; Dalby et al., 2010). Similar intensive agricultural management in systems with soil disturbance and continuous monocrop systems have resulted in reduced soil organic matter (SOM) (Liu et al., 2006). SOM acts as a source of inorganic nutrients such as nitrogen (N), microbial food, ion exchange surface, and is a factor in root development (Allison, 1973), and is essential in maintaining soil quality in continuous cropping systems (Reeves, 1997). Organic sources of N are lost during the removal of litter-fall. Every 100 kg of macadamia harvested also removes around 405 g of N that was partitioned into the nut (Nagao and Hirae, 1992). It is also suggested that leaves are a significant N sink (Fletcher et al., 2009) and storage pool (Stephenson et al., 1986; Huett et al., 2001) for macadamia. The removal of these leaves from the agroecosystem can lead to a significant loss of N from the macadamia cropping system.

Fertilizer application is currently required to keep nutrient concentrations at the recommended levels in macadamia orchards. The conventional use of fertilizers is suggested in serious harmful environmental effects. Before the 1920’s atmospheric N_2O levels did not commonly exceed 280 ppb. Levels have risen, due mainly to agriculture, to new highs of 328 ppb (EPA, 2016). Additionally, the excess use of fertilizer to ensure proper yields can lead to runoff, causing nutrient pollution, promoting eutrophication, and harmful algal blooms downstream (Heisler et al., 2008), while not improving overall production (Stephenson and Gallagher, 1989). Stakeholders in Hawai‘i see replacing imported fertilizers with locally produced organic sources as a high priority, and that sourcing local inputs ranked third on their list of barriers to increasing local crop production (Radovich et al., 2009; Ahmad et al. 2016).

A potential source of local soil amendments in macadamia is the left-over husk, shell, and wood chips from harvest, pruning, and tree removal. Mulches can to improve indicators related to soil health that improve growth and yield. Gao et al. (2009) observed a 53.6% nutrient use efficiency increase in straw mulch compared to a 36% increase with no mulch after three years. Wood chip mulch in tea olive (*Osmanthus fragrans)* improved root activity by 24.5% and chlorophyll content 6.47% compared to no mulch (Xue et al., 2016). Porter et. al (2005) observed similar increases in macadamia root growth and yield from macadamia husk mulch applications compared to a control but indicated that a lag period of two to three years may be necessary to produce results. A similar length of time was also necessary to observe increases in leaf N concentrations in ‘Giles’ pecan (*Carya illinoiensis*) (Smith et al., 2000). Pecan mulch treatment increased leaf N concentrations 86% over mowing treatment in newly established ‘Loring’ peach (*Prunus persica*) trees in one year, did not increase yield, and also led to higher tree mortality due to excess rainfall (Stafne et al., 2009). After 18 months, macadamia husk compost increased microbial biomass by 300% compared to bare soils in macadamia soils (Cox et al., 2004). Increased microbial activity assists N cycling and availability for plants (Raviv

1998). Additional and novel soil amendments are now being used in macadamia production and could be assessed for efficacy.

Biochar is another available option for farmers on Hawaiʻi Island. Biochar can have a varied impact on yield. A meta-study conducted in 2011 found that in 51 studies evaluating the effects of biochar, as much as a 28% decrease and a 100% increase in yield can occur (Jeffery et al.). Biochar can also be a direct source of plant available N (Zheng et al., 2013) and change other soil properties that impact nutrient availability and plant growth and yield (Baiamonte et al., 2015; Laird et al., 2010; Liang et al. 2010). Biochar application methods usually call for the incorporation of biochar into soil but in perennial cropping systems where soil is not tilled the surface application of biochar occurs.

Effective microorganisms are a novel method of organic management employed in Hawaiʻi, specifically the culturing of indigenous microorganisms (IMO). Macadamia growers are interested in seeing results from this practice (Pers. comm., Nathan Trump, June 15, 2016). IMO is a non-selective collection of naturally occurring microorganisms, usually done by placing steamed rice outdoors in the agroecosystem. The organisms that colonize this rice are then cultured using a carbohydrate source, water, and a growth medium. This culture is diluted with water and sprayed onto the plants and soils in production systems. These cultures of unknown populations are difficult to study, so using a product of known population for research is one solution. A product called EM1®, developed by Teruo Higa, was used to emulate indigenous microorganisms. EM1 is composed of *Lactobacillus casei*, a lactic acid bacterium (LAB). LAB's function with soil amendments by consuming carbohydrates in biomass, speeding up the decomposition rates. LAB can increase yields in plant growth. Olle (2016) noted yield increases of 43% in peas in Europe, but Mayer et al. (2010) detected no significant increase in crop yields over 4 years. Similar studies have noted increases in yield (Iwaishi, 2000; Khaliq et al., 2006). The effects of EM1 on macadamia production have not been assessed. Soil profiling, also called fraze mowing is a practice generally used in turf grass management but is being employed in macadamia orchards in Australia and now Hawaiʻi. Soil profiling is defined as the mechanical removal and displacement of up to the top .5 to 5 cm of soil surface. The soil is removed from the inter-rows of macadamia orchards and deposited into the rows directly under the center of the macadamia canopies. The effects of soil profiling in Hawaiʻi have not been studied.

The objective of this study was to compare the effects of new and novel soil amendment treatments on yield and quality, leaf SPAD values, and root growth in mature macadamia orchards. The soil amendment treatments were expected to increase SPAD values, root growth, and potentially yield over the control treatment within a year time frame.

3.3 Materials and methods

3.3.1 Site

Field experiments were conducted between August 2016 and March 2018. The site of research is located in the Kohala region in the Northwest portion of the island of Hawaiʻi, at a ~700 acre macadamia orchard. Two soil orders are present in this region, andisol and inceptisol. The two sites were selected based on uniformity of macadamia variety. Site 1 soil is composed of ainakea medial silty clay loam with a 3-12% slope. Site 2 is composed of kohala silty clay with a 3-12% slope. Site 1 was certified organic in 2012 and is managed organically while site 2 is managed conventionally. Average monthly temperatures range from 20.7° C to 24.3° C. Average annual rainfall is 141.32 cm with monthly averages ranging from 7.16 cm to 17.74 cm (Giambelluca et al., 2014). The macadamia cultivar being used in the experiment is HAES 508 'Kakea', and is a scion grafted onto seedlings of the cultivar HAES 660 'Keaau'.

3.3.2 Experimental design and implementation

The experiment was set up as a randomized complete block design. Four blocks were selected at each site. In each block the six treatments were randomly assigned to the plots. Six treatments were applied in March of 2017 (Table 3.1) on plots consisting of four trees in a linear arrangement, totaling 192 trees. During the experiment, all management practices were uniform among all blocks. The previous season's husk was developed into partially decomposed mulch by being formed into windrows following the harvest season (February 2016). Wood chip mulch was sourced from a neighbor farm and consisted of multiple species of trees. Biochar was sourced from Pacific Biochar (Pahoa, HI). The EM product was sourced from Terra Granix (Alto, TX). Soil amendments were applied to plots in March 2017. Baseline properties of all solid mulch inputs are presented in Table 3.2.

3.3.3 Yield and quality sampling

Tree yield as wet-in-husk weight was collected during three harvest sampling times in August, October, and December 2017. During each sampling period a subsample of 20 nuts per plot were collected. Total weights were taken for wet-in-husk and wet-in-shell. Nuts were dried following procedures by Wall and Gentry (2007) to 10% moisture. Total dry shell weight was taken. Individual nuts were weighed for dry shell weight, kernel weight, and assessed for oil content using a float test, assessed for wholeness of the kernel, and assessed for defects. Kernels that float and have no defects are counted as No. 1 grade. The following calculation is used to determine kernel recovery rate.

$$\% \text{ No 1. kernel recovery} = \frac{\text{Wt. total No 1. grade kernels}}{\text{Wt. total dried nuts-in-shell}} \times 100 \qquad \text{Eq. (2)}$$

This equation gives the total weight of saleable kernel compared to the amount of dry-in-shell nut.

3.3.4 SPAD value sampling

15 measurements were taken from two randomly selected trees in each plot totaling 30 measurements per plot, using a Minolta SPAD-502 chlorophyll meter. The measurement amount is following protocols for tissue sampling in Macadamia (Hirae, 1976). Leaf selection criteria was mature, healthy leaves on the second whorl back from the mature (no longer green and not flushing) tip of a branch exposed to full sun. Measurements were taken near the midpoint on the midrib of each leaf sample. Samples were taken monthly beginning before the treatments are applied in March 2017 until February 2018.

3.3.5 Root growth sampling

Root samples were collected in February 2017, June 2017, and February 2018. Mulch was gently moved from the soil surface before collection. Porter et al. (2005) found that macadamia root growth occurred in mulch substrate. A soil sample of 10 cm in depth and 2 cm width volume=31.42 cm^3) was collected using a soil corer (2 cm diameter) from two locations in each plot, from the inter-row and within the row. Samples were collected at the same distance as the drip line of the individual tree's canopy. Soil samples were soaked in water for 30 minutes. The solution was drained into a mesh sieve measuring 250 micrometers. Roots were separated and placed back into water solution for soaking. The process was repeated until roots were free from soil and clean. Roots were then placed in metal drying boats and dried at 70° C for 72 hours. Roots are then weighed for dry weight/volume. Proteoid roots were separated from the total root sample and weights were recorded.

3.3.6 Partial cost benefit analysis

A partial cost benefit analysis was conducted using cost information from the farmer. Material and application costs are estimated at a per acre basis for all mulch treatments, after adjusting for hourly application rates. Hourly equipment depreciation was estimated using annual depreciation divided by estimated hours of annual use. Yields were estimated for wet-in-shell weight by taking average wet-in-husk harvest data by treatment and converting to wet-in-shell using wet-in-shell subsample data averaged by treatment. Yields were estimated for acre using mean tree yields for treatments and multiplying by an estimated number of trees per acre if planted at 15' x 30' spacing. The weights per acre were adjusted based on average kernel recovery rates for the treatments. When purchasing macadamia, processors adjust the weight and amount paid based on kernel recovery rate. The industry standard in Hawai‘i for purchasing macadamia is based on a 30% kernel recovery. Yields with a kernel recovery higher or lower than 30% resulted in an adjusted weight based on confidential calculations. These calculations were used to adjust the weights for the yields in this study. Price per pound was obtained from USDA final season estimates (USDA, 2017). Profit was based off of a partial cost estimate using only the costs of applying the treatment including soil amendment cost. All other costs would be even among treatments. The profit is not meant to be an estimate of what the actual profit would be, due to the exclusion of additional operating costs.

3.3.7 Statistical analysis

All data were subjected to analysis using JMP Pro version 13.1 (SAS Institute Inc., NC, USA). Levene's tests were performed to assess equal variance. Shapiro-Wilks test was used to test for normal distribution. The null hypothesis for equal variance was rejected for total yield ($p = 0.0278$). Data were transformed using $\log_{10}$ transformation. The null hypothesis for equal variance was not rejected after data transformation ($p = 0.1138$). Two-way ANOVA using mixed model personality was used to test for effects and interactions with block included as a random effect. Post Hoc Tukey HSD tests and student's t-tests were performed for comparisons of means.

3.4 Results and discussion

3.4.1 Yield and quality

Treatment affected total wet-in husk yield per tree (Table 3.3). The soil profiling treatment had a mean yield of 86.6 kg (Table 3.4) per tree and was higher than all other treatments with the exception of the husk treatment (70.3 kg/tree). Kernel recovery rate was not affected by treatment (Table 3.3). The control had the highest kernel recovery rate at 31.7% but was not statistically significantly different from other treatments (Table 3.4). Sampling period affected overall kernel recovery but there was no interaction with treatment (Table 3.3). August had a mean kernel recovery rate of 33% and was higher than the October and December harvests both of which had mean kernel recovery rates of 29.2% (Fig. 3.1). Dry kernel weight was similar among treatments (Table 3.3). Location and month both had an effect on dry kernel weights (Table 3.3). Dry kernel weights were higher for site 2 compared to site 1 and increased at both sites from the August sampling period to the October sampling period and decreased during the December sampling period (Fig. 3.2).

Soil profiling is a technique that destructively removes a portion of the soil surface. Macadamia root structure exists very close to the surface of the soil (Firth et al., 2003). The acute destruction of root biomass can cause water stress in plants, which tends to increase abscisic acid (ABA) production, an endogenous compound that is associated with abscission in plants including flowers and fruit (Ohkuma et al., 1963). The soil profiling may have had a negative effect on shoot growth, through disrupting abscisic acid-ethylene dynamics (Sharp and LeNoble, 2002), while having little effect on yield due to the high density of flowers and macadamia's unique ontogenesis. The soil profiling treatment occurred in March of 2017, during the primary flowering and before fruit development for macadamia. Root pruning may have caused some additional flower abscission, but this most likely did not have a negative effect on yield. Macadamia can produce more than 10,000 racemes with 100-300 flowers per raceme, but generally only 0.3% of these flowers develop into fruit (Urata, 1954; Ito, 1980). As long as flowers are not physically removed the sheer number of flowers can act as a buffer against flower abscission. The root pruning could have affected yield through disrupting the competition between vegetative and reproductive growth. The restriction of vegetative growth in apples reduced fruit abscission and increased yield (Quinlan and Preston, 1971). Alternatively, root pruning in apple trees reduced shoot growth but also reduced yield in apple trees (Mcartney and Belton, 2011). In an experiment on macadamia, post-pruning shoot growth reduced yield compared to those with shoots removed (McFayden et al., 2011). If the soil profiling did have a negative effect on shoot growth and did not negatively affect flowering, this could have caused a larger amount of carbohydrates to be assimilated towards the fruit during development. The lack of competition from vegetative growth would have diverted a higher than normal source of carbohydrates, explaining why the soil profiling treatment resulted in the greatest yields.

This study suggests harvesting time plays an important role in kernel recovery rates. Kernel recovery was higher in earlier months. Rainfall most likely had an effect on kernel recovery rate. Weather data was used from a weather station adjacent to the experimental sites in Kapa'au (Fig.'s 3.3 & 3.4). Precipitation was relatively low during the summer of 2017 (Fig. 3.3). October and November of 2017 had the highest precipitation during the whole study period at 19.8 cm and 26.6 cm respectively. This is a relatively great deal of precipitation compared to the total precipitation in July (0.7 cm) and August (7.5 cm). Humidity was also higher during October (79.1%) and November 2017 (81.8%) compared to the summer months (Fig. 3.4) although not as drastic a contrast as the differences between the total precipitation for the months. Considering harvesting times were equally spaced out and the nuts from the August harvest time resided in the field the longest, precipitation is the most likely cause of reduced kernel recovery rate. Mold can be a significant issue in lowering kernel recovery rate, and excessive moisture exacerbates this issue by promoting mold growth. Excess moisture also has an accelerating effect on rancidification (Woodroof, 1979), another factor which reduces kernel recovery rate. The effect of location on dry kernel weights may be explained by the management practices. Organically grown apples resulted in lower fruit weights to their conventionally grown counterparts (do Amarate et al., 2008; Roussos and Gaspartos, 2009). There may also be underlying microclimatic differences in the sites that are causing this disparity in kernel weight that are not accounted for.

3.4.2 SPAD value

SPAD values were affected by location, sampling period, and treatment interactions (Table 3.3). SPAD values followed a similar ontogenetic trend depending on location. For site 1, SPAD values expressed a pronounced negative trend immediately in April 2017 in concert with flowering and fruit development but almost none of these decreases were significant (Fig. 3.5). The only treatment to significantly decrease in April 2017 was the husk treatment in Site 1. SPAD values remained relatively low throughout the summer. Treatments in site 2 did not experience the same trend, with most SPAD values decreasing very little from March 2017 to April 2017 while some increased (Fig. 3.6). The mean SPAD values for trees that's received the wood chip treatment in site 2 declined from July to November 2017, a trend that was not observed in any other treatment. There was also a cyclic pattern in the fluctuation of SPAD values. This trend was more or less apparent in every treatment in site 1 and partially in site 2, with SPAD values experiencing a negative trend and a subsequent positive trend occurring bimonthly until a positive trend occurred in late fall.

For site 1, SPAD values did not significantly change from pretreatment to posttreatment for any of the treatments (Table 3.5). Despite the lack of significant change over a year, some treatments did differ from each other in February of 2018, which may lead to a wider range of differences between the treatments on longer term studies. The husk, husk+EM1, and wood chip treatments had higher SPAD values than the control (Table 3.5). In March of 2017, neither the husk+EM1 nor wood chip treatments were significantly different than the control. Despite the treatments resulting in similar changes in SPAD values, and none of the treatments causing a large increase in SPAD values from pre- to post-treatment, the husk, husk+EM1, and wood chip treatments were successful in increasing SPAD values over the control by post-treatment. For site 2, SPAD values did increase from pre- to post-treatment for the husk+EM1 and soil profiling treatments (Table 3.5). Both treatments had an average SPAD value of 50.4 in the March 2017 sampling time and increased by a value of 3.3 for the husk+EM1 treatment and 3.5 for the soil profiling treatment. This implies that only the husk+EM1 and soil profiling treatments significantly increased SPAD values within a year time frame in the conventionally managed site.

Changes in SPAD values are related to changes in leaf N and chlorophyll content and are affected by N availability in the soil. The addition of mulch treatments could cause N immobilization in the soil reducing plant available N. N immobilization would be greater in the organic plot based on the assumption that organic systems have more microbial biomass and activity than conventional systems (Lori et al., 2017). The same assumption could be used to explain the SPAD value trend in the wood chip treatment in site 2 (Fig. 3.6). Leaf water content can also affect SPAD measurements, with lower leaf water content correlating to higher SPAD values (Martinez and Guiamet, 2003). An explanation for the differences between the marked reduction in SPAD values at site 1 from March 2017 to April 2017 could be differences in water holding capacity at site 1 compared to site 2. This could be due to differences in the soil series or management practices. Time of day is also a potential source of variation in the samples, due to a change in leaf water concentration and its effect on SPAD (Chang and Robinson, 2002). Researchers found that organic management in coffee orchards resulted in higher water holding capacity compared to conventional orchards (Velmourougane, 2016). The increased water holding capacity in concert with mulch application could result in higher water availability in these treatment plots. The increase in SPAD values in the trees receiving the husk+EM1 treatments might be attributed to the increased proteoid root growth on these trees (Table 3.6).

3.4.3 Root growth

Total root biomass

Total root biomass was affected by sampling period and treatment had an interaction with sampling period for (Table 3.3). The mixed model suggests a location interaction with treatment, however post hoc tests suggest no treatment differences between locations. There were almost no significant differences in total root biomass by volume between treatments for all sampling periods with the exception of February 2018. The husk+EM1 resulted in higher total root biomass compared to the wood chip treatment in February of 2018 (Table 3.6). Some treatments did result in an increase over time. The husk+biochar treatment resulted in an increase of 0.21 g and the husk+EM1 treatment resulted in an increase of 0.20 g from February 2017 to February 2018 (Table 3.6). The wood chip treatment resulted in a decrease in total root biomass of -0.04 g, but this was not statistically significant.

Proteoid root biomass

Proteoid root biomass was affected by treatment, sampling period and the interaction between sampling period and treatment (Table 3.3). The mean proteoid root weight by volume was significantly higher for the husk+EM1 treatment compared to all other treatments in February of 2018 (Table 3.6). This treatment was also the only treatment to result in significant changes from February 2017 to February 2018 with an increase of 153.7 mg. The wood chip treatment resulted in a decrease in proteoid root biomass of -8.4 mg and the control resulted in a small increase of +.6 mg, but these changes were not statistically significant. The husk, husk+biochar, and soil profiling treatments resulted in large and statistically insignificant increases.

Proteoid root proportion

Treatment had an effect on proportion of samples with proteoid roots and month had an interaction with treatment (Table 3.3). The proportion of samples with proteoid root biomass was affected by treatment for the month of February 2018 (Table 3.6). The mean proportion of samples with proteoid root biomass was significantly higher in husk+EM1 treatment, at 87.5%, than every other treatment except for the soil profiling treatment (50%). The husk+EM1 and soil profiling treatments both resulted in higher proportions of proteoid roots compared to the control (6.3%). The control and wood chip treatments resulted in decreases in proportions of proteoid roots, but these decreases were not statistically significant.

The husk+EM1 treatment affected root growth in macadamia significantly. It was the only treatment to significantly increase total root biomass, proteoid root biomass, and proteoid root initiation. Previous reports suggest that proteoid roots are influenced by microbial inoculation of the soil of macadamia (Malcolm, 1979) and soil microorganisms are responsible for the formation of proteoid roots in other proteaceous genera (Lamont and McComb, 1974). Proteoid roots also occur in *Lupinus* species, and their formation requires sugar signaling (Zhou, 2008). Incomplete polysaccharide metabolism and exopolysaccharide production by *L. casei* could have an effect on sugar availability and therefore signaling of proteoid root production in the soils of macadamia orchards. Additionally, the use of molasses as a food source for *L. casei* may have had the additional effect of providing a sugar source for proteoid root signaling. *L. casei* has not been identified as one of the microorganisms responsible for the promotion of proteoid roots in macadamia in any available previous study.

3.4.4 Partial cost benefit analysis

Based on the results of the partial cost benefit analysis (Table 3.7), husk mulch and wood chip mulch are the cheapest soil amendment options at $64.50 per acre. The husk mulch resulted in the second highest estimated gross profit per acre. The soil profiling treatment is the second cheapest option at $89.00 an acre and resulted in the highest profit per acre at $12,951.44 per acre based on partial cost deductions. Both the combinatorial treatments are the most expensive treatment options, due to the fact that the biochar and EM1 both were applied separately from the mulch. These costs could be reduced if the additional inputs were combined with the mulch before applying the mulch to the orchards. The benefits of organic agriculture extend beyond yield and include yield stability, biodiversity, soil and water quality, climate change mitigation, farmer and worker health and quality of life, to a varying degree, all of which are context-dependent (Seufert and Ramankutty, 2017).

3.5 Conclusion

This study suggests that soil amendment treatments can affect yield, SPAD values, and root growth in mature macadamia trees within a year time frame. Soil profiling was the only treatment to have a higher yield than the control and could be explored as a yield increasing management practice to exercise judiciously. It is recommended that long-term effects of repeated soil profiling be studied. Mulches may cause a short-term reduction in leaf SPAD values in an organic system however husk+EM1 has the potential to increase SPAD values and implicitly leaf tissue N concentrations in both organic and conventional systems. The increase in SPAD values in trees that received the husk+EM1 treatment may be attributed to the increase in proteoid growth in those trees. Husk+EM1 dramatically increased root growth compared to all other treatments, especially proteoid root growth. Proteoid roots are very effective in nutrient and water uptake and have the potential to improve the plants ability to access these resources. Fresh wood chips have the potential to decrease both total root growth and proteoid root growth. Economically, soil profiling is the most profitable treatment choice at a profit of $12,951.44/acre after deducting the cost of treatment. The wood chip treatment had the lowest profit at $9,317.46/acre. In summary, soil profiling can be a short-term method to improve yield and husk+EM1 can improve leaf N content and root growth. Husk+EM1 is not recommended as a stand-alone treatment with a N concentration of 1.1% (Table 3.2). Fresh wood chips had a negative effect on yield, SPAD values, and root growth and are not recommended as an effective treatment. The relationship between proteoid root growth and changes in leaf N concentrations would be a potential area of study to improve nutrient use efficiency in macadamia. The long-term effects of these treatments on macadamia and soil health is worth evaluation as well.

3.6 Tables

Table 3.1- Soil amendment treatments with application method, equipment used, and application rates as applied to macadamia trees in March 2017 in Kapa'au, HI.

Amendment	Application rate	Method
Control [z]	---	---
Macadamia husk mulch	1.4 m^3, 5 cm depth, 3 m radius	compost spreader
Macadamia husk mulch + biochar	mulch- 1.4 m^3, 5 cm depth, 3 m radius biochar- 0.14 m^3, 1 cm depth, 3 m radius	compost spreader
Macadamia husk mulch + EM1	mulch- 1.4 m^3, 5 cm depth, 3 m radius EM1- 10 L/tree, 1:1:1000 EM:Molasses:Water	Compost spreader 100 L sprayer
Soil profile	0.7 m^3, 2.5 cm depth, 3 m radius	soil profiler
Wood chip Mulch	1.4 m^3, 5 cm depth, 3 m radius	compost spreader

[z] Application rate and method are not applicable for control plots receiving no treatment.

Table 3.2- Baseline properties of solid mulch inputs applied to macadamia trees in March 2017 in Kapa'au, HI.

Input	N (%)	C (%)	pH	EC (mS/cm)
Wood chip	0.31	45.36	6.69	1.68
Husk	1.10	46.6	7.68	0.98
Biochar	0.31	61.17	9.50	10.5

Table 3.3- Two-Way ANOVA full factorial reports for significance of effects and interactions for location, month, and treatment on plant growth and yield response variables for macadamia trees receiving six soil amendment treatments applied to macadamia trees in March 2017 in Kapa'au, HI.

Source of variation [z]	Total yield [z]	Kernel recovery rate	Dry kernel weight	Total dry root weight	Proteoid dry root weight	Proteoid root proportion	Leaf SPAD value
Location (L)	0.1806	0.1459	**<0.0001**	0.0574	0.4243	0.5855	**0.0059**
Month (M)	-	**<0.0001**	**<0.0001**	**0.0007**	**<0.0001**	0.1266	**<0.0001**
Treatment (T)	**0.0002** [y]	0.4003	0.4109	0.8258	**<0.0001**	**0.0001**	**<0.0001**
L*M	-	0.0917	0.0921	0.9273	0.3453	0.964	**<0.0001**
L*T	0.1074	0.9242	0.2597	**0.0072**	0.7456	0.2912	**<0.0001**
M*T	-	0.458	0.2146	**0.0390**	**<0.0001**	**0.001**	**<0.0001**
L*M*T	-	0.6959	0.1188	0.7354	0.3642	0.3651	**<0.0001**

[z] Month and month by interactions not applicable to total yield.
[y] Bold text indicates a statistically significant difference with a $P \leq 0.05$.

Table 3.4- Mean total wet-in-husk yield per tree, mean kernel recovery rate, and mean dry kernel weight, averaged by treatment, of macadamia trees receiving six soil amendment treatments for the 2017-2018 harvest season.

Treatment	Mean yield [z] (kg/tree)	Mean kernel recovery (%)	Mean Dry Kernel wt. (g)
Control	67.6[a]	31.7[NS]	2.63[NS]
Husk	70.3[ab]	29.7	2.68
Husk+biochar	66.8[a]	30.9	2.72
Husk+EM1	66.3[a]	30.8	2.73
Soil profile	86.6[b]	29.9	2.73
Wood chip	60.5[a]	30.8	2.62

[z] Means in the same column followed with matching letter(s) are not significantly different. NS= No significant difference found among treatments.

Table 3.5- Mean SPAD values for macadamia trees receiving six soil amendment treatments for the pre-treatment sampling time March 2017 and final sampling February 2018 and change from pre-treatment to final sampling.

Leaf SPAD value						
Sampling Period			Site 1			
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
March 2017 [z]	51.6^{ab}	55.2^{c}	51.0^{a}	52.7^{ab}	54.0^{bc}	54.0^{bc}
February 2018	51.4^{a}	54.2^{b}	53.1^{ab}	54.6^{b}	53.8^{ab}	55.5^{b}
Change [y]	-0.2	-1	+2.1	+1.9	-0.2	+1.5
			Site 2			
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
March 2017	51.3^{NS}	50.9	51	50.4	50.4	49.5
February 2018	51.5^{abc}	52.8^{abc}	51.2^{ac}	53.7^{ab}	53.9^{b}	51.1^{c}
Change	+0.2	+1.9	+0.2	**+3.3**	**+3.5**	+1.6

[z] Data are separated by site and means in the same row in the same site with matching letter(s) are not significantly different. NS= No significant difference found among treatments.
[y] Bold text indicates a statistically significant difference between March 2017 and February 2018 with a $P \leq 0.05$.

Table 3.6- Mean dry biomass weights of total root, proteoid root, and proportion of proteoid root presence by sampling period for macadamia trees receiving six soil amendment treatments and change from pre-treatment to final sampling.

Sampling period			Mean total root mass by volume (g)			
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
February 2017	0.25[NS]	0.25	0.16	0.23	0.25	0.26
June 2017	0.27[NS]	0.28	0.31	0.27	0.22	0.32
February 2018 [z]	0.27[ab]	0.31[ab]	0.37[ab]	0.43[a]	0.37[ab]	0.22[b]
Change [y]	+0.02	+0.06	**+0.21**	**+0.20**	+0.12	-0.04
			Mean proteoid root weight by volume (mg)			
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
February 2017	2.0[NS]	3.2	1.6	2.6	3.6	10.5
June 2017	3.4[NS]	7.6	0.0	7.9	15.2	8.5
February 2018	2.6[a]	22.2[a]	20.9[a]	156.3[b]	18.3[a]	2.1[a]
Change	+0.6	+19.0	+19.3	**+153.7**	+14.7	-8.4
			Proportion of samples with proteoid roots (%)			
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
February 2017	18.8[NS]	25.0	12.5	31.3	31.3	43.8
June 2017	25.0[NS]	31.3	0	25.0	43.8	37.5
February 2018	6.3 [c]	43.8[bc]	25.0[bc]	87.5[a]	50.0[ab]	12.5[bc]
Change	-12.5	+18.8	+12.5	**+56.2**	+18.7	-31.3

[z] Data are separated by response variable and means in the same row in the same response variable with matching letter(s) are not significantly different. NS= No significant difference found among treatments.
[y] Bold text indicates a statistically significant difference with a $P \leq 0.05$ between February 2017 and February 2018.

Table 3.7- Partial cost benefit analysis showing estimated cost and benefit per acre for five soil amendment treatments applied in a macadamia orchard in Kapa'au, HI.

Application	Husk	Husk +biochar	Husk +EM1 [x]	Soil profile	Wood chip	No treatment
Material cost/acre ($)	25.00	100.00	7.00	0.00	25.00	0.00
Labor cost/hr ($)	22.00	22.00	22.00	22.00	22.00	0.00
Fuel cost/hr ($)	6.50	7.00	5.75	7.50	6.50	0.00
Equipment depreciation/hr ($)	11.00	11.00	19.50	15.00	11.00	0.00
Number of acres treated/hr [z]	1	6.5	0.25	0.5	1	0
Cost/acre ($)	64.50	106.15	132.91	89.00	64.50	0.00
+ Husk (biochar and EM1) ($) [y]	----	64.50	64.50	----	----	----
Total cost/acre ($)	64.50	170.65	197.41	89.00	64.50	0.00
Yield benefits						
Estimated wet-in-shell yield/tree (lb)	95.19	90.46	89.78	117.29	81.92	91.52
Estimated yield/acre (lb)	9,232.99	8,774.32	8,708.80	11,377.43	7,946.33	8,877.37
kernel rec. rate adjustment yield/acre (lb)	9,140.66	9,037.55	8,941.03	11,339.51	8,158.23	9,380.43
Price per lb (2017) ($)	1.15	1.15	1.15	1.15	1.15	1.15
Revenue/acre ($)	10,511.76	10,393.18	10,282.18	13,040.44	9,381.96	10,787.49
Profit after deducting treatment costs ($) [w]	10,447.26	10,222.53	10,084.77	12,951.44	9,317.46	10,787.49

[z] This number is based on how many acres can be treated per hour for each treatment. This is based on speed of the equipment and other operating limitations that limit the application efficiency. This is used to adjust labor hours, fuel cost, and equipment depreciation from per hour to per acre.

[y] Husk mulch, soil profiling, and wood chip mulch not mixed in combination with husk.

[x] Cost/acre for EM1 treatment based on 2.5 hours preparation and clean up and 1.5 hours applying for one acre. Labor costs are calculated at 4 hours and fuel and equipment depreciation are calculated at 1.5 hours.

[w] Profit is partially calculated. Only treatment costs are deducted. Complete operating costs are considered the same for all treatments and not included in this partial profit calculation.

3.7 Figures

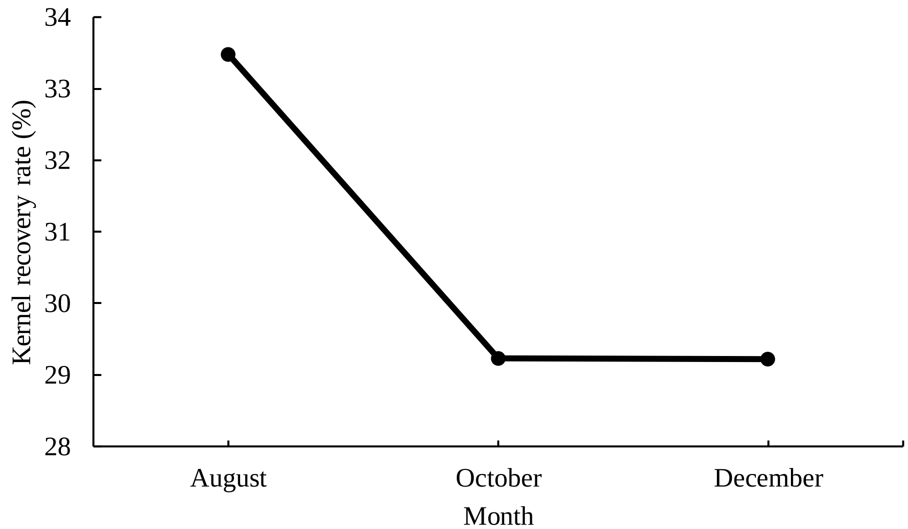

Figure 3.1- Mean kernel recovery rate averaged by sampling period for macadamia receiving six soil amendment treatments for the 2017-2018 harvest season.

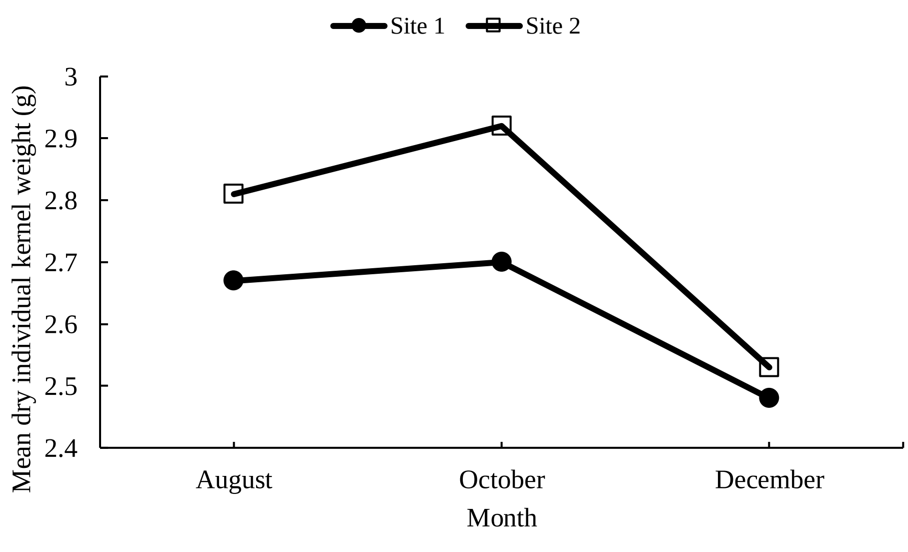

Figure 3.2- Mean dry individual kernel weight averaged by location and by month for macadamia receiving six soil amendment treatments for the 2017-2018 harvest season.

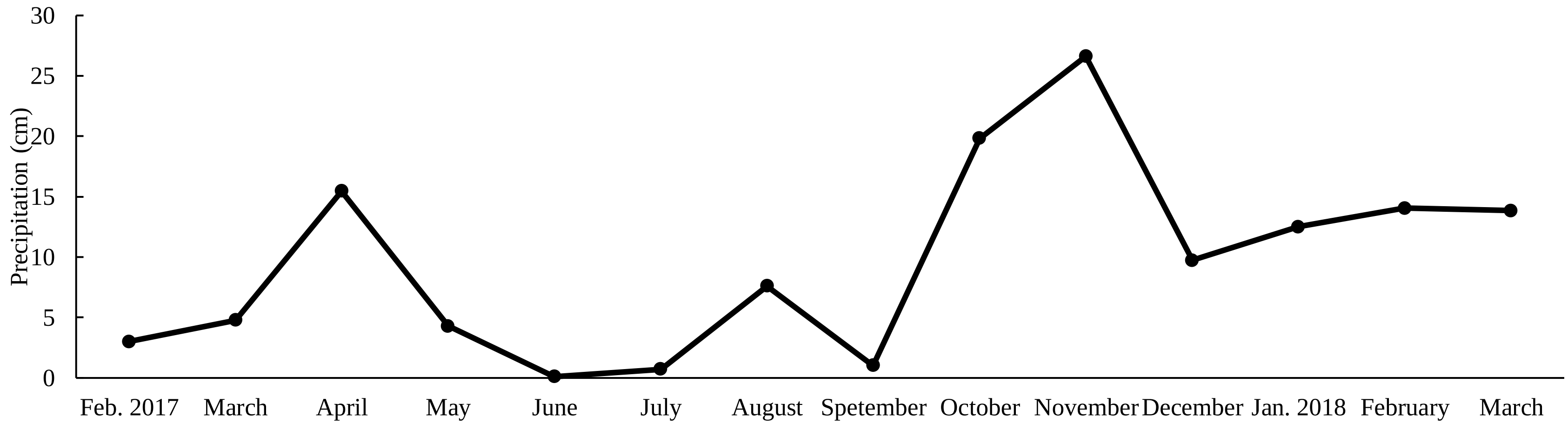

Figure 3.3 Total monthly precipitation from February 2017 to March 2018 as collected by a weather station adjacent to experimental sites in Kapa’au, HI.

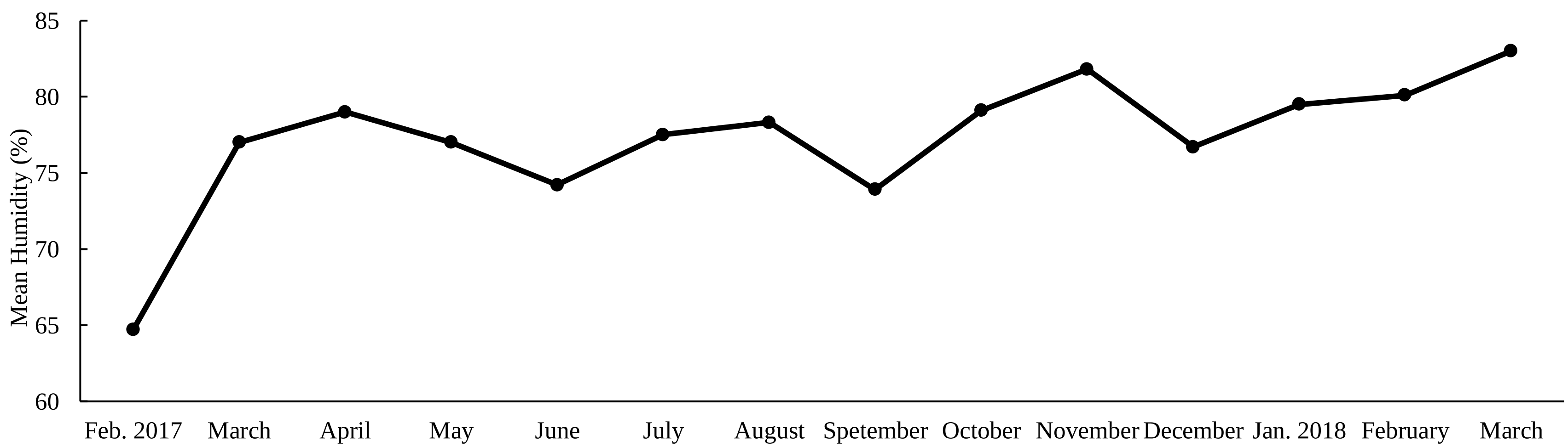

Figure 3.4- Mean humidity by month from February 2017 to March 2018 as collected by a weather station adjacent to experimental sites in Kapa’au, HI.

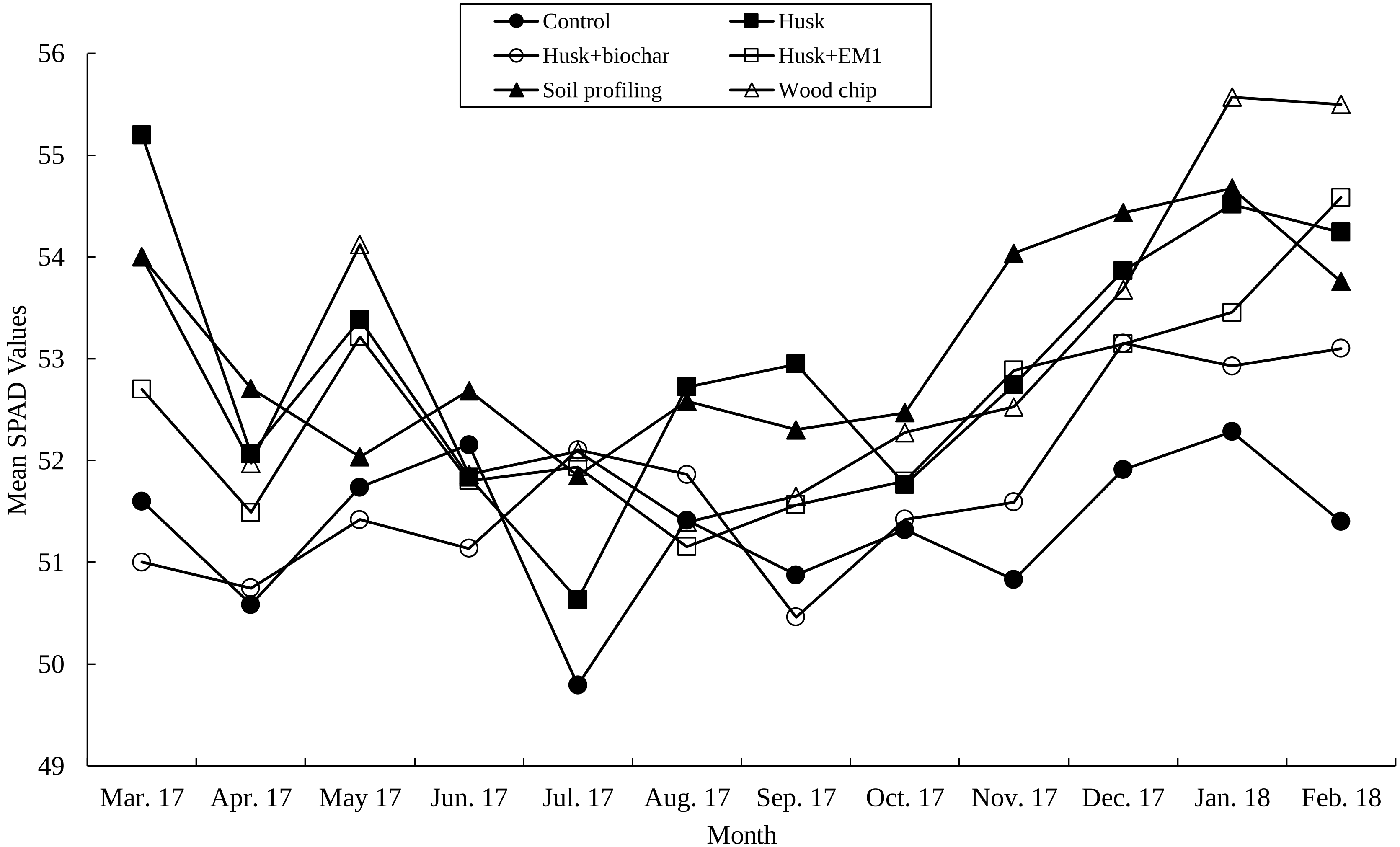

Figure 3.5- Mean SPAD readouts averaged by treatment and sampling period showing changes in SPAD values for each treatment from March 2017 to February 2018 for macadamia trees receiving six soil amendment treatments at Site 1[z].

[z] Certified organic in 2012.

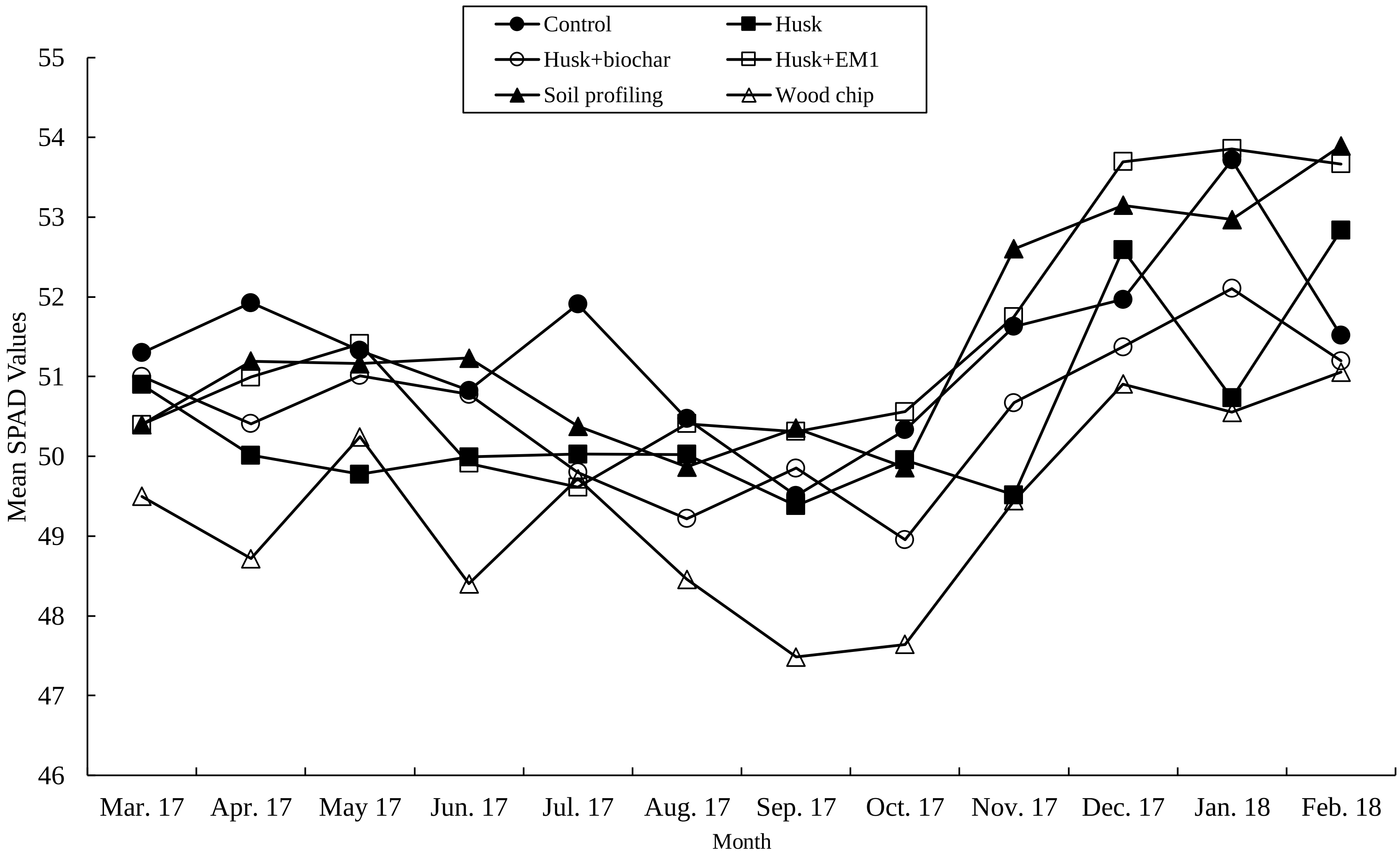

Figure 3.6- Mean SPAD readout averaged by treatment and sampling period showing changes in SPAD values for each treatment from March 2017 to February 2018 for macadamia trees receiving six soil amendment treatments at Site 2 z.

[z] conventionally managed.

3.8 References

Ahmad, A.A., Radovich, T., Nguyen, H.V., Uyeda, J., Arakaki, A., Cadby, J., Paull, R., Sugano, J., Teves, G. (2016). Use of Organic Fertilizers to Enhance Soil Fertility, Plant Growth, and Yield in a Tropical Environment. In: M.L. Larramendy and S. Soloneski, (eds.), *Organic Fertilizers-From Basic Concepts to Applied Outcomes*. Chapter 4, p: 85-108. http://www.intechopen.com/books/organic-fertilizers-from-basic-concepts-to-applied-outcomes.

Allison, F.E. (1973). *Soil Organic Matter and its Role in Crop Production*. Amsterdam. Elsevier.

Baiamonte, G., Pasquale, C., Marsala, V., Cimo, G., Alonzo, G., Crescimanno, G., Conte, P. (2014). Structure alteration of a sandy-clay soil by biochar amendments. *Journal of and Soils Sediments*. DOI 10.1007/s11368-014-0960-y

Cox, J., Van-zwieten, L., Ayres, M., Morris, S. (2004). Macadamia husk compost improves soil health in sub-tropical horticulture. *Super Soil: 2004 3rd Australian New Zealand Soils Conference*. University of Sydney, Australia.

Chang, S., Robinson, D. (2003). nondestructive and rapid estimation of hardwood foliar nitrogen status using the SPAD-503 chlorophyll meter. *Forest Ecology and Management* 181, 331-338.

Dalby, T., Cox, J., Morris, S. (2010). Harvest equipment and soil erosion in a macadamia orchard. *19th World Congress of Soil Science, Soil Solutions for a Changing World*, 1-6 August 2010, Brisbane, Australia.

do Amarante, C.V.T., Steffens, C.A., Luiz Mafra, A., Albuquerque, J.A., (2008). Yield and fruit quality of apple from conventional and organic production systems. *Pesq. Agropec. Bras. Brasilia*. 43, 333–340.

Firth, D., Whalley, R., Johns, G. (2003). Distribution and density of the root system of macadamia on krasnozem soil and some effects of legume groundcovers on fibrous root density. *Australian Journal of Experimental Agriculture. 43*(5), 503-514.

Fletcher, A., Rennenberg, H., Schmidt, S. (2009). Nitrogen partitioning in orchard grown *Macadamia integrifolia. Tree Physiology*. 30, 244-256.

Gao, Y., Li, Y., Zhang, J., Liu, W., Dang, Z., Cao, W., Qiang, Q. (2009). Effects of mulch, N fertilizer, and plant density on wheat yield, wheat nitrogen uptake, and residual soil nitrate in a dryland area of China. *Nutrient Cycling in Agroecosystems.* DOI 10.1007/s10705-009-9252-0

Giambelluca, T., Chen, Q., Frazier, A., Price, J., Chen, Y., Chu, P., Eischeid, J. Delparte, D. (2013). Online Rainfall Atlas of Hawai‘i. *Bulletin of the American Meteorological Society*. 94, 313-316.

Hirae, H. (1976). Leaf and soil analysis in macadamia. *Hawaii Macadamia Producers' Assoc. 16th Annual Proceedings.* p. 65.

Huett, D.O., B.J. Gogel, M.N. Meyers, C.A. McConchie, L.M. McFayden and S.C. Morris. (2001). Leaf nitrogen and phosphorus levels in macadamias in response to canopy position and light exposure, their potential as leaf-based shading indicators, and implications for diagnostic leaf sampling protocols. *Australian Journal of Agriculture Research.* 52, 513–522.

Iwaishi, S., (2000). Effect of organic fertilizer and effect of microorganisms on growth, yield and quality of paddy-rice varieties. *Journal of Crop Production.* 3, 269-273.

Ito, P. (1980). Effect of style removal on fruit set in macadamia. *HortScience*. 15,520–521.

Jusoh, M., Manaf, L., Latiff, P. (2013). Composting of rice straw with effective microorganisms (EM) and its influence on compost quality. *Irnanian Journal of Environmental Health Sciences & Engineering*. *10*(17).

Kahliq, A., Abbasi, M.K., Hussain, T. (2006). Effects of integrated use of organic and inorganic nutrient sources with effective microorganisms (EM) on seed cotton yield in Pakistan. *Bioresource Technology*. 97, 967-972.

Laird, D., Fleming P., Davis D., Horton R., Wang B., Karlen D. (2010). Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. *Geoderma*. 158, 443–449.

Lamont, B., McComb, A. (1974). Soil microorganisms and the formation of proteoid roots. *Australian Journal of Botany*. *22*(4), 681-688.

Liang B., Lehmann J., Sohi S., Thies J., O'Neill B., Trujillo L., Gaunt J., Solomon D., Grossman J., Neves E., Luizão F. (2010). Black carbon affects the cycling of non-black carbon in soil. *Organic Geochemistry*. 41,206–213.

Liu, X., Herbet, S., Hashemi, A., Zhang, X., Ding, G. (2006). Effects of agricultural management on soil organic matter and carbon transformation- a review. *Plant Soil Environment*. 52, 531-543.

Lori, M., Symnaczik, S., Mader, P., De Deyn, C., Gattinger, A. (2017). Organic farming enhances soil microbial abundance and activity- A meta-analysis and meta-regression. PLoS ONE 12(7): e0180442. https://doi.org/10.1371/journal.pone.0180442

Malcolm, H. (1979). Proteoid roots help macadamia nut trees. *Ag. Gazette of New South Wales. 90*(1), 42-43.

Martinez, D., Guiamet, J. (2004). Distortion of the SPAD-502 chlorophyll meter readings by changes in irradiance and leaf water status. *Agronomie*. *24*(1), 41-46.

Mcartney, S., Belton, R. (1992). Apple shoot growth and cropping responses to root pruning. *New Zealand Journal of Crop and Horticultural Science.* 20, 383-390.

Mcfayden, L., Robertson, D., Sedgley, M., Kristiansen, P., Olesen, T. (2011). Post-pruning shoot growth increases fruit abscission and reduces stem carbohydrates and yield in macadamia. *Annals of Botany. 107*(6), 993-1001.

Nagao, M., Hirae, H. (1992). Macadamia: Cultivation and Physiology. *Critical Reviews in Plant Science, 10*(5), 441-470.

Ohkuma K, Lyon J., Addicott F. (1963). Abscisin II, an abscission-accelerating substance from young cotton fruit. *Science.* 142, 1592–1593.

Olle M., Williams I. (2013). Effective microorganisms and their influence on vegetable production – a review. *Journal of Horticultural Science & Biotechnology*. *88*(4), 380 – 386.

Porter, G., Yost, R., Nagao, M. (2005). the Application of Macadamia Nut Husk and Shell Mulch To Mature Macadamia Integrifolia To Improve Yields, Increase Nutrient Utilization, and Reduce Soil P Levels. *Western Nutrient Management Conference*. *6*, 226–233. Salt Lake City, UT.

Quinlan, J., Preston, A. (1971). The influence of shoot competition on fruit retention and cropping of apple trees. *Journal of Horticultural Science.* 46, 525-534.

Radovich, T. J. K., Cox, L. J., Hollyer, J. R. (2009). Overview of Organic Food Crop Systems in Hawaiʻi. College of Tropical Agriculture and Human Resources. SA-3.

Raviv, M. (1998). Horticultural uses of composted material. *Acta Horticulturae*. 469, 225-234.

Reeves, D. (1997). The role of soil organic matter in maintaining soil quality in continuous cropping systems. *Soil & Tillage Research.* 43, 131-167.

Roussos, P., GAspartos, D. (2009). Apple tree growth and overall fruit quality under organic and conventional orchard management. *Scientia Horticulturae*. *123*(2), 247-252.

Seufert, V., Ramankutty, N. (2017). Many shades of gray- The context-dependent performance of organic agriculture. *Science Advamnces.* 3, 1-14.

Sharp, R., LeNoble, M. (2002). ABA, ethylene and the control of shoot and root growth under water stress. *Journal of Experimental Botany*. *53*(366), 33-37.

Smith, M., Carroll, B., Cheary, B. (2000). Mulch improves pecan tree growth during orchard establishment. *HortScience*. *35*(2), 192-195.

Stafne, E., Rohla, C., Carroll, B. (2009). Pecan shell mulch impact on 'Loring' peach tree establishment and first harvest. *HortTechnology*. *19*(4), 775-780

Stephenson, R. and B.W. Cull. (1986). Flushing patterns of macadamia trees in south east Queensland. *Acta Horticulturae.* 175, 49– 53.

Stephenson, R. and E.C. Gallagher. (1989). Timing of nitrogen application to Macadamias. E. Reproductive growth, yield and quality. *Australian Journal of Experimental Agriculture.* 29, 581–585.

United States Department of Agriculture. (2017). *Noncitrus Fruits and Nuts 2014 Summary.* ISSN: 1948-2698. USDA, National Agricultural Statistics Service.

Urata U. (1954). Pollination requirements of macadamia. *Hawaiian Agricultural Experiment Station Technical Bulletin*. No. 22.

Velmourourgane, K. (2016). Impact of organic and conventional systems of coffee farming on soil properties and culturable microbial diversity. *Scientifica*. http://dx.doi.org/10.1155/2016/3604026.

Woodroof, J.G. (1979). *Tree Nuts: Production, Processing, Products, second ed*. AVI Publisher, Westport.

Zheng, H., Wang, Z., Deng, X., Zhao, J., Luo, Y., Novak, J., Herbert, S., Xing, B. (2013). Characteristics and nutrient values of biochars produced from giant reed at different temperatures. *Bioresource Technology*. 130, 463–471.

Zhou, K., Yamagishi, M., Osaki, M., Masuda, K. (2008). Sugar signaling mediates cluster root formation and phosphorous starvation-induced gene expression in white lupin. *Journal of Experimental Botany*. *59*(10), 2749-2756.

CHAPTER 4

THE EFFECTS OF SOIL AMENDMENTS ON SOIL CARBON, NITROGEN, pH, AND ELECTRICAL CONDUCTIVITY IN MACADAMIA ORCHARDS

4.1 Abstract

Soil management in macadamia orchards can lead to orchard soil degradation and yield loss. Farmers in Hawaiʻi have shown interest in sourcing local inputs that can partially defray the use of imported inputs. Several new soil amendment options have been identified and their effects on soil health parameters were assessed. Husk mulch, husk+biochar, husk+effective microorganisms (EM1®), soil profiling, and wood chip mulch were applied in a randomized complete block experimental design and soil Carbon, Nitrogen, pH, and electrical conductivity (EC) were assessed over a year-long study in a certified organic orchard (site 1) and a conventional orchard site (site 2). Most soil response variables had some interaction with location, with the exception of EC. Total N was not significantly increased by any treatment. NO_3- and NH_4^+ dynamics were affected by treatments over time. Husk treatments resulted the greatest increases in NO_3- concentrations and were among the least decreases in NH_4^+ concentrations. pH was only significantly increased in site 2 for the husk, husk+biochar, and soil profiling treatments. EC was increased by the husk+biochar treatment in site 1 by 0.42 mS/cm and all three husk treatments increased soil EC in site 2. Soil C was significantly increased by husk and husk+EM1treatments in site 1. Husk treatments have the potential to increase NO_3- while not causing a significant reduction in NH_4^+ as well as increase soil C. Potential issues with husk mulch use, particularly in combination with biochar or EM1 are increases in pH and EC. Mulches generally have more influential effects long-term and results of their effects over a longer period of time would be valuable.

4.2 Introduction

Soil management in macadamia (*Macadamia integrifolia* Maiden & Betche) orchards can lead to several serious issues affecting soil quality and plant available nutrients. Particular management involves using machines to sweep or blow the orchard floors clean of all debris to facilitate nut harvest. This leads to exposed soil surface and reduced groundcover which can cause soil loss and reduced soil quality (Dalby et al., 2010; Reid, 2002). This management practice removes most non-anthropogenic sources of soil organic matter (SOM). A large portion of nitrogen (N) in macadamia is partitioned in the vegetative and reproductive tissues that fall as litter-fall (Huett et al., 2001; Nagao and Hirae, 1992; Stephenson et al., 1986). The removal of litter-fall and harvesting practices removes these sources. For maturing orchards this can pose a serious risk to orchard soil health and ultimately productivity. Conventional fertilizer is the traditional practice to alleviate loss of N. Conventional N fertilizer use generally results ins a reduction in soil pH (Bunemann et al., 2006) and increase electrical conductivity (EC). This can pose a problem by lowering pH below the recommended 5.8 to 6.2 range for macadamia production in Hawai‘i (Fox, 1974; Tamimi et al. 1994). Fertilizer production and use leads to higher atmospheric N_2O, a greenhouse gas (EPA, 2016). Furthermore, excess fertilizer inputs cause fertilizer runoff, eutrophication and harmful algal blooms (Heisler et al., 2008). Stakeholders in agriculture in Hawaii prioritize reduced reliance on imported conventional fertilizers and sourcing local inputs as a high priority (Radovich, 2009).

Soil quality can be described as the degree of fitness for a specific use. SOM, soil N, pH and EC are common indicators of soil quality for macadamia production. SOM is a direct source of nutrients, improves soil structure, and is a source of food for soil organisms (Allison, 1973). Nitrogen is the element needed in highest concentrations in macadamia leaf tissue (Nagao and

Hirae, 1992) and N is a proposed cause of yield variability in macadamia (Stephenson et al., 1997). Extreme pH and EC values can have detrimental effects on macadamia (Nagao and Hirae, 1992). Organic matter (OM) should be a regular input to improve soil quality in soils lacking OM (King, 1990; Theng, 1991). OM inputs generally result in high mean changes in soil C increases in short term experiments (Paul et al., 1996). Use of mulches can significantly improve soil C concentration in orchards (Porter et al., 2005; Sanchez et al., 2003; Youkhana and Idol, 2009; Xue, 2016) and N concentrations (Youkhana and Idol, 2009; Xue, 2016) in tropical conditions. EC is an important consideration for agricultural production. Soils treated with mulches may increase in EC (Hueso-Gonzalez et al., 2014). Bezborodov et al. (2010) found that this increase in EC was 20% higher in non-mulched treatments.

Mulches and composts are among the most common OM inputs. The effects of various mulch applications in macadamia orchards have been the focus of previous studies (DeFrank et al., 1989; Firth et al. 1994; Porter et al., 2005) though these studies did not focus on N. Several soil amendments sourced locally have been identified as inputs for improving orchard soils including biochar, effective microorganisms (EM), and a soil profiling practice (also called fraze mowing). These can be used in combination with traditional mulch sources such as macadamia husk mulch and wood chip mulch. Biochar often increases pH in acidic soils, increases soil C, and has varying effects on soil N (Chan et al., 2007; Major et al., 2010). Short-term in pot experiments using EM resulted in increases in total N and specifically increases in NO_3^- with no significant change in pH (Canbolat et al., 2005). Soil profiling is a novel practice in macadamia orchards adopted from turfgrass management and is defined as the mechanical removal and displacement of up to the top .5 to 5 cm of soil surface. The soil is removed from the inter-rows of macadamia orchards and deposited into the rows directly under the center of the macadamia canopies. No available research on the effects of soil profiling on macadamia orchards in Hawai‘i is available. The objective of this study was to evaluate the effect of soil amendments on soil C, N, pH, and EC. Mulches were expected to increase soil C and N over the control. While EC was expected to increase in mulched plots this increase was anticipated to not be significant compared to the control. The Biochar treatment was the only treatment expected to increase EC by a notable amount. pH was expected to increase in all plots subjected to treatments.

4.3 Materials and Methods

4.3.1 Site

Field experiments were conducted between August 2016 and March 2018. The site of research is located in the Kohala region in the Northwest portion of the island of Hawai‘i, at a ~700 acre macadamia orchard. Two soil orders are present in this region, andisol and inceptisol. The two sites were selected based on uniformity of macadamia variety. Site 1 soil is composed of ainakea medial silty clay loam with a 3-12% slope. Ainakea series is defined as a strong fine and medium granular structure, well drained moderately rapid permeability and strongly acidic (pH 4.4) within the first 25 cm. Site 2 is composed of kohala silty clay with a 3-12% slope as well. Kohala series is defined as a moderate fine granular structure, well drained moderately rapid permeability and slightly acidic (pH 6.3) within the first 18 cm. Site 1 was certified organic in 2012 and is managed organically. Site 2 is managed conventionally. Average monthly temperatures range from 20.7° C to 24.3° C. Average annual rainfall is 141.32 cm with monthly averages ranging from 7.16 cm to 17.74 cm (Giambelluca et al., 2014). The macadamia cultivar being used in the experiment is HAES 508 ‘Kakea’, and is a scion grafted onto seedlings of the cultivar HAES 660 ‘Keaau’.

4.3.2 Experimental design and implementation

The experiment was set up as a randomized complete block design. Four blocks were selected at each site. In each block the six treatments were randomly assigned and applied in March of 2017 (Table 4.1) on plots consisting of four trees in a linear arrangement, totaling 192 trees. During the experiment, all management practices were uniform among all blocks. The previous season's husk was developed into partially decomposed mulch by being formed into windrows following the harvest season (February 2016). Wood chip mulch was sourced from a neighbor farm and consisted of multiple species of trees. Biochar was sourced from Pacific Biochar (Pahoa, HI). The EM product was sourced from Terra Granix (Alto, TX). Soil amendments were applied to plots in February 2017.

4.3.3 Soil sampling

Soil samples were collected in August 2016 before treatment application, June 2017, and February 2018. Samples were collected from the row and inter-row in each plot. Mulch was displaced down to a clean soil surface before collecting samples. A sample of soil was collected using a hand trowel from the surface of the soil down to a depth of 5 cm. Soil samples were homogenized and held at 0° C after collection.

4.3.4 Nitrogen analysis

Samples were sent to University of Hawai‘i at Mānoa's Sustainable Farming Systems Laboratory (Honolulu, HI). Plant available N was estimated using ion-selective Vernier electrodes and a LabQuest interface (Vernier Software, Beaverton, OR). Five (5) grams of soil were weighed and placed in a 50 mL falcon tube and 30 mL of deionized water was added to the falcon tube, a 1:7 dilution ratio. Samples were shaken for one hour and filtered through quantitative filter paper. Vernier ion-selective electrodes were used to measure nitrate (NO_3-) and ammonium (NH_4^+) ions according to manufacturer's recommendations. The electrodes were soaked in standard high solutions (100 mg/L) for 30 minutes and calibrated to high (100 mg/L) and low (1 mg/L) standard solutions before each use. Calibration curves were developed by reading standard solutions at nine increments from 1 mg/L to 100 mg/L. Samples were brought up to ambient temperature (25° C) prior to analyses. The electrodes were individually placed into the filtered solution and data was collected. The calibration curves were used to adjust electrode readings at each sampling period.

Total N was measured by gas chromatography. Samples were dried for 24 hours at 105° C and sieved through an 833 micrometer sieve. Samples were analyzed using a Costech 4010 elemental analyzer at University of Hawaiʻi at Hilo Analytics Lab.

4.3.5 pH and EC analysis

pH and EC were analyzed from filtered solutions prepared during nitrogen analysis using a digital meter (Model 98129, Hanna Instruments, Woonsocket, RI). The meter was calibrated for EC using a standard solution of 12.88 mS/cm and pH standard solutions of 7.01 and 4.01.

4.3.6 Carbon analysis

Direct estimation of C was assessed by gas chromatography. Samples were dried for 24 hrs at 105° C and sieved through an 833 micrometer sieve. Samples were analyzed using a Costech 4010 elemental analyzer at University of Hawaii at Hilo Analytics Lab.

4.3.7 Statistical analysis

All data were subjected to analysis using JMP Pro version 13.1 (SAS Institute Inc., NC, USA). Levene's tests were performed to assess equal variance. Shapiro-Wilks test was used to test for normal distribution. The null hypotheses for equal variance failed to be rejected for soil C (p = 0.0072), NO_3- (p = 0.0005), NH_4^+ (p = 0.0037), and EC (p = 0.0087). Soil C, NO_3-, and EC data were transformed using $\log_{10}$ transformation and NH_4^+ data was transformed using square-root transformation. All null hypotheses for equal variance were rejected after transformation for soil C (p = 0.0733), NO_3- (p = 0.1724), EC (p = 0.1168), and NH_4^+ (p = 0.0504). Two-way ANOVA using mixed model personality was used to test for effects and interactions with block included as a random effect. Post Hoc Tukey HSD tests were performed for comparisons of means.

4.4 Results and discussion

4.4.1 Soil nitrogen

Mean total soil N (%) was affected by sampling period, and location had an interaction with treatments (Table 4.2). Mean total soil N for all treatments for both sites did not significantly differ from each other in August of 2016. Total N concentration decreased from August 2016 to June 2017 for most treatments with the exception of the control in site 2 and wood chip treatment in site 1 (Table 4.3). Nearly all treatments resulted in an increase in total N from August 2016 to February 2018, but these increases were not significant. For site 2, the husk+biochar treatment was lower than the wood chip treatment in February 2018 (Table 4.3). The only treatment to decrease in total N was the husk+biochar treatment at site 2 with a reduction of -0.01%. The husk+EM1 treatment for site 1 resulted in the highest increase of +0.18% N, while at site 2 the husk and the wood chip treatment resulted in the highest increases at a shared +0.11% N. These changes in the plots with husk+biochar, husk+EM1, husk, and wood chip treatments were not statistically significant though.

Mean soil NO_3- concentrations (mg/L) were affected by location and sampling period, but not treatment (Table 4.2). NO_3- concentrations decreased from August 2016 to June 2017 with the exception of the husk+EM1 and soil profiling treatments in site 2 (Table 4.4). All treatments except for the control increased NO_3- concentrations from August 2016 to February 2018 for site 1 (Table 4.4), however no treatments were significantly higher than the control in February 2018. For site 1 the husk treatment resulted in the greatest increase in mean soil NO_3- (+16.59 mg/L) concentration. For site 2, only the husk and husk+biochar treatments increased NO_3- concentrations from August 2016 to February 2018. Similar to site 1, NO_3- concentrations did not significantly differ between treatments for the sampling period of February 2018 (Table 4.4), and the husk treatment resulted in the greatest increase in mean soil NO_3- (+28.42 mg/L).

Mean soil NH_4^+ concentrations (mg/L) were affected by treatment and sampling period, and sampling period had an interaction with treatments (Table 4.2). NH_4^+ at site 1 generally increased from August 2016 to June 2017 with the exception of the wood chip treatment which resulted in a small decrease (Table 4.5). Site 2 had no general trend from August 2016 to June 2017. For both sites, the control, soil profiling, and wood chip treatments resulted in decreases in NH_4^+ concentrations (Table 4.5). The wood chip treatment resulted in the greatest decrease in soil NH_4^+ at -5.88 mg/L at site 1 and -6.16 mg/L at site 2 (Table 4.5). For site 1, the husk+biochar treatment was higher than the control, soil profiling, and wood chip treatments for the sampling period of February 2018. For site 2, the husk and husk+biochar treatments were both higher than the control, soil profiling, and wood chip treatments for the sampling period of February 2018. (Table 4.5).

Total soil N remained similar from pretreatment to posttreatment for all treatments. This is not surprising as all of the treatment inputs had very low baseline N% (Table 4.2). The treatments did appear to affect NO_3- and NH_4^+ dynamics. The husk treatments in general resulted in the greatest increases in NO_3- concentrations and buffered against considerable decreases in NH_4^+ concentrations. The husk treatment in particular resulted in the greatest increases in NO_3- at both sites and was among the highest total N increases. This increase from the husk mulch is not surprising as the highly decomposed husk treatment had the highest N concentration in the baseline sampling of mulch inputs (Table 4.2). The husk+biochar treatment did not increase total N substantially and in site 2 was the only treatment to slightly decrease total N. This lack of response from the husk+biochar treatment could be due to biochar's ability to alter soil N dynamics and adsorb NO_3- and NH_4^+ (Clough et al., 2013) as it leached from the husk mulch. Due to its C:N ratio of 146:1 (Table 4.2) the wood chip mulch was expected to decrease total N through microbial immobilization; this did not occur, however total N, NO_3-, and NH_4^+ changes for the wood chip treatment were closely similar to the control. The difference in the N concentration change for the wood chip in site 1 (+0.01%) compared to site 2 (+0.11%) may be explained by a great deal of research that states organic systems have more microbial biomass and activity than conventional systems (Lori et al., 2017) and this would cause greater N immobilization. This would only be a reasonable explanation if the N immobilizing microbes were colonizing the mulch substrate. The soil profiling treatment resulted in similar patterns to the control treatment for soil N response variables. This is reasonable based on the fact that the soil profiling treatment is essentially just a displacement of orchard soil.

4.4.2 Soil pH and EC

Mean soil pH was not affected by treatment and was affected by sampling period, and location had an interaction with sampling period and treatment (Table 4.2). pH increased from August 2016 to June 2017 and generally decreased from June 2017 to February 2018 at site 1. pH increased continuously between these sampling periods at site 2 (Table 4.6). None of the treatments in site 1 significantly increased pH pretreatment to posttreatment. At site 2, the husk, husk+biochar, and soil profiling treatments increased pH from August 2016 to February 2018 (Table 4.6). pH for the husk and husk+biochar treatments were 6.09, and 6.12 respectively in February 2018

The baseline pH for the husk and biochar amendments were 7.68 and 9.50 respectively and the wood chip mulch was 6.69 (Table 4.2). This is reflected in the pH changes of the soil under these treatments. The increase in the pH under the soil profiling treatment can be attributed to potentially high pH in the soil and debris that was displaced from the interrows into the rows. Site 1, the organically managed site, had a more buffered response in pH changes to the treatments. The organic site had a slightly higher mean pH (5.66) than the conventional site (5.50) at pretreatment (Fig. 4.5). This difference along with the slightly higher soil C concentration at in the organic site (6.04%) compared to 5.75% at the conventional site (Fig. 4.6) could explain some of the buffering effect in the organically managed site. SOM is a major influence on pH buffering due to its contribution to cation-exchange-capacity and its provision of weak acids (Magdoff and Weil, 2004). The differences in mean pH and mean soil C at pretreatment were not significant between the two sites, however the trend indicated a higher increase in soil C and more buffered pH at the organic site (Figs. 4.1 & 4.2). Additionally, there was more nitrification occurring at Site 1 compared to Site 2. Nitrification can lower pH by releasing Hydrogen ions during the process.

Mean soil EC was affected by treatment, sampling period, and treatment had an interaction with sampling period (Table 4.2). EC generally increased throughout the year-long study in all treatments. Mean EC increased for the husk, husk+biochar, husk+EM1 from pretreatment to posttreatment (Table 4.6). The husk+biochar treatment resulted in the highest posttreatment EC value of 0.84 mS/cm and was significantly higher than the control, soil profiling, and wood chip treatments all at 0.56 mS/cm in February 2018 (Table 4.6).

The baseline EC for the husk and biochar amendments were 0.98 mS/cm and 10.5 mS/cm respectively and the wood chip EC was 1.68 mS/cm (Table 4.2). These baseline values are reflected in the EC changes for the husk+biochar treatment resulting in significant increases in soil EC. The wood chip treatment, however, did not result in large or significant increases, as was expected.

4.4.3 Soil carbon

Mean total soil C (%) was affected by sampling period, and location had an interaction with treatments, but treatments had no significant effect on soil C (Table 4.2). C concentration increased at both sites for all treatments from the pretreatment sampling period to February 2018 (Table 4.7). The husk and husk+EM1 treatments at site 1 increased soil C concentrations over the year-long study (Table 4.7). The husk treatment increased C from 6.30% to 9.65%, and the husk+EM1 treatment increased C from 5.78% to 11%. The husk treatment also increased C concentrations by the greatest amount in site 2 but this difference was not significant (Table 4.7).

The highly decomposed husk treatment could be easily introduced into the soil within the first year due to its decomposed state causing increases in soil C. The husk+biochar treatment at both sites did not result in comparatively high increases, potentially because the layer of biochar physically prevented the incorporation of the mulch into the soil. This suggests that biochar could potentially slow down the incorporation of organic matter into the soil in the short term in macadamia orchard soils if applied as a contiguous layer between mulch and the soil surface.

4.5 Conclusion

Total soil N was similar among treatments at the end of the study. The soil amendments baseline concentrations of N are not high compared to that of conventional fertilizer. At N concentrations below 2% (Table 4.1) large increases in total N concentrations in the soil were not expected. Nitrification was occurring at greater rates for all treatments except for the control at the organic site, and the husk and husk+biochar treatments at the conventional site. The nitrification process has the potential to convert ammonium to nitrate and lead to nitrate leaching and losses in total soil N. The nitrogen cycle is affected by changes into the agroecosystem, including inputs (Sainju, 2017). The most important factors in controlling nitrification are NH_4^+ concentrations and oxygen availability in the soil (Robertson, 1989). The mulches possibly influenced these factors by influencing water relations, NH_4^+ concentrations, soil temperature, soil structure, and respiration in the soil. Nitrification may be an undesirable occurrence and the effects of these soils amendments on nitrification should be considered if nitrate leaching is a primary concern for growers.

Nitrification can also decrease soil pH through the release of H^+ ions. At site 2, the treatments that caused the greatest increase in NO_3-, also resulted in the greatest increases in pH. The influence of the husk and husk+biochar treatments on increasing pH at site 2 had to be greater than the rate of nitrification. The buffering capacity of the organically managed site should be taken into account when considering that mulch soil amendments generally increase pH, a potentially harmful effect of their use. Organically managed macadamia orchards may have a greater resilience to pH increases caused by mulching and would not require pH adjustments through the use of inputs if the trend of increasing pH is a chronic issue. The husk+biochar treatment did increase soil EC more than expected. The high baseline EC of the husk+biochar treatment probably caused the increases in soil EC under this treatment. Using biochar repeatedly should be done with caution, and the baseline EC of any biochar product should be evaluated before application. Soil C increases were not substantial; most likely it takes longer than a year to result in greater increases compared to not mulching due to the slow incorporation of organic matter into the soil in uncultivated orchards soils. There is potential for husk treatments both alone and combined with effective microorganisms to increase soil C in organically managed macadamia orchards while not negatively affecting pH or EC. The long-term effects of these soil amendment treatments on soil properties in macadamia orchards deserves further review. Understanding additional soil physical and chemical properties will help expose the driving factors behind the changes in the soil properties discussed in this study.

4.6 Tables

Table 4- Baseline properties of solid mulch inputs applied to macadamia trees in March 2017 in Kapa’au, HI.

Input	N (%)	C (%)	pH	EC (mS/cm)
Wood chip	0.31	45.36	6.69	1.68
Husk	1.10	46.6	7.68	0.98
Biochar	0.31	61.17	9.50	10.5

Table 5- Two-Way ANOVA full factorial reports for significance of effects and interactions for location, month, and treatment on soil NO_3^-, NH_4^+, total N, pH, EC, and total C response variables for macadamia trees receiving six soil amendment treatments.

Source of variation	DF	NO_3-	NH_4^+	%N	pH	EC	%C
Location (L)	1	**0.0252** [z]	0.7244	0.1807	0.3572	0.0611	0.2119
Month (M)	2	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Treatment (T)	5	0.1215	**<0.0001**	0.0962	0.909	**<0.0001**	0.2477
L*M	2	**0.0015**	0.271	0.2288	**<0.0001**	0.3409	0.2983
L*T	5	0.8872	0.0232	**0.0080**	**0.0193**	0.6758	**0.0196**
M*T	10	0.0624	**<0.0001**	0.0678	0.3439	**0.0210**	0.1237
L*M*T	10	0.8378	0.4469	0.4383	0.7034	0.6698	0.3382

[z] Bold text indicates a statistically significant difference with a $P \leq 0.05$.

Table 6- Mean Soil N concentrations (%) by sampling period for soil under macadamia trees receiving six soil amendment treatments and change from pre-treatment to final sampling.

Sampling period	Nitrogen concentration (%)					
	Site 1					
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
August 2016	0.5^NS	0.51	0.52	0.48	0.51	0.46
June 2017	0.46^NS	0.46	0.49	0.46	0.46	0.47
February 2018	0.54^NS	0.63	0.54	0.66	0.57	0.56
Change [z]	+0.04	+0.12	+0.01	+0.18	+0.06	+0.1
	Site 2					
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
August 2016	0.47^NS	0.45	0.42	0.47	0.51	0.48
June 2017	0.48^NS	0.43	0.41	0.46	0.41	0.47
February 2018 [y]	0.56^ab	0.56^ab	0.41^a	0.50^ab	0.52^ab	0.59^b
Change	+0.09	+0.11	-0.01	+0.03	+0.01	+0.11

[z] Bold text indicates a statistically significant difference with a $P \leq 0.05$ between August 2016 and February 2018.
[y] Data are separated by site and means in the same row in the same site with matching letter(s) are not significantly different. NS= No significant difference found among treatments.

Table 7- Mean NO_3- concentrations (mg/L) by sampling period for soil under macadamia trees receiving six soil amendment treatments and change from pre-treatment to final sampling.

	NO_3^- (mg/L)					
Sampling period			Site 1			
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
August 2016 [z]	9.94^{NS}	10.78	12.18	10.50	9.87	11.41
June 2017	6.09^{NS}	8.26	6.23	7.42	9.52	10.50
February 2018	19.39^{NS}	27.37	25.83	23.73	20.02	23.10
Change [y]	+9.45	**+16.59**	**+13.65**	**+13.23**	**+10.15**	**+11.69**
			Site 2			
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
August 2016	13.72^{NS}	12.18	13.93	12.81	12.88	14.42
June 2017	11.55^{NS}	11.76	12.04	14.56	15.40	11.20
February 2018	23.66^{NS}	40.60	37.03	22.40	23.03	23.59
Change	+9.94	**+28.42**	**+23.10**	+9.59	+10.15	+9.17

[z] Data are separated by site and means in the same row in the same site with matching letter(s) are not significantly different. NS= No significant difference found among treatments.

[y] Bold text indicates a statistically significant difference with a $P \leq 0.05$ between August 2016 and February 2018.

Table 8- Mean NH_4^+ concentrations (mg/L) by sampling period for soil under macadamia trees receiving six soil amendment treatments and change from pre-treatment to final sampling.

Sampling period	NH_4^+ (mg/L)					
	Site 1					
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
August 2016	7.07^{NS}	8.12	6.65	8.40	6.93	7.63
June 2017	10.92^{NS}	8.82	14.84	14.84	9.10	7.35
February 2018 [z]	2.03^{a}	4.62^{ab}	6.51^{b}	5.18^{ab}	1.75^{a}	1.75^{a}
Change [y]	**-5.04**	-3.50	-0.14	-3.22	**-5.18**	**-5.88**
	Site 2					
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
August 2016	6.86^{NS}	7.42	11.97	7.35	6.58	7.35
June 2017	5.74^{a}	12.60^{ab}	16.87^{b}	9.24^{ab}	5.81^{a}	7.70^{a}
February 2018	1.84^{ab}	5.74^{c}	6.58^{c}	4.83^{bc}	0.84^{a}	0.98^{a}
Change	**-5.04**	-1.47	-5.60	-2.52	**-5.74**	**-6.16**

[z] Data are separated by site and means in the same row in the same site with matching letter(s) are not significantly different. NS= No significant difference found among treatments.
[y] Bold text indicates a statistically significant difference with a $P \leq 0.05$ between August 2016 and February 2018.

Table 9- Mean pH and EC (mS/cm) by sampling period for soil under macadamia trees receiving six soil amendment treatments and change from pre-treatment to final sampling.

pH						
Sampling period		Site 1				
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
August 2016 [z]	5.74[NS]	5.59	5.62	5.89	5.61	5.48
June 2017	5.88[NS]	5.71	5.65	5.75	5.75	5.74
February 2018	5.54[NS]	5.63	5.63	5.78	5.60	5.66
Change	-0.20	+0.04	+0.01	-0.11	-0.01	+0.18
		Site 2				
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
August 2016	5.48[NS]	5.52	5.53	5.47	5.43	5.58
June 2017	5.81[NS]	5.91	5.80	5.74	5.92	5.84
February 2018	5.86[NS]	6.09	6.12	5.89	5.99	5.88
Change [y]	+0.38	**+0.57**	**+0.59**	+0.42	**+0.56**	**+0.30**

EC (mS/cm)						
Sampling period	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
August 2016	0.42[NS]	0.42	0.42	0.42	0.35	0.49
June 2017	0.49[NS]	0.56	0.63	0.56	0.49	0.49
February 2018	0.56[a]	0.70[ab]	0.84[b]	0.70[ab]	0.56[a]	0.56[a]
Change	+0.14	**+0.28**	**+0.42**	**+0.28**	+0.21	+0.07

[z] Data are separated by site and/or response variable and means in the same row in the same site and/or response variable with matching letter(s) are not significantly different. NS= No significant difference found among treatments.
[y] Bold text indicates a statistically significant difference with a $P \leq 0.05$ between August 2016 and February 2018.

Table 10- Mean soil C concentrations (%) by sampling period for soil under macadamia trees receiving six soil amendment treatments and change from pre-treatment to final sampling.

Carbon concentration (%)						
Sampling period		Site 1				
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
August 2016 [z]	5.98^{NS}	6.30	6.54	5.78	6.19	5.50
June 2017	5.77^{NS}	5.70	6.39	5.89	5.73	6.05
February 2018	7.40^{NS}	9.65	8.23	11.00	8.08	7.98
Change [y]	+1.42	**+3.35**	+1.69	**+5.22**	+1.89	+2.48
		Site 2				
	Control	Husk	Husk+biochar	Husk+EM1	Soil profile	Wood chip
August 2016	5.48^{NS}	5.70	5.27	5.96	6.21	5.90
June 2017	6.27^{NS}	5.98	5.34	5.84	5.05	6.20
February 2018	7.95^{NS}	8.80	6.07	7.33	7.07	8.72
Change	+2.47	+3.10	+0.80	+1.37	+0.86	+2.82

[z] Data are separated by site and means in the same row in the same site with matching letter(s) are not significantly different. NS= No significant difference found among treatments.
[y] Bold text indicates a statistically significant difference with a $P \leq 0.05$ between August 2016 and February 2018.

4.7 Figures

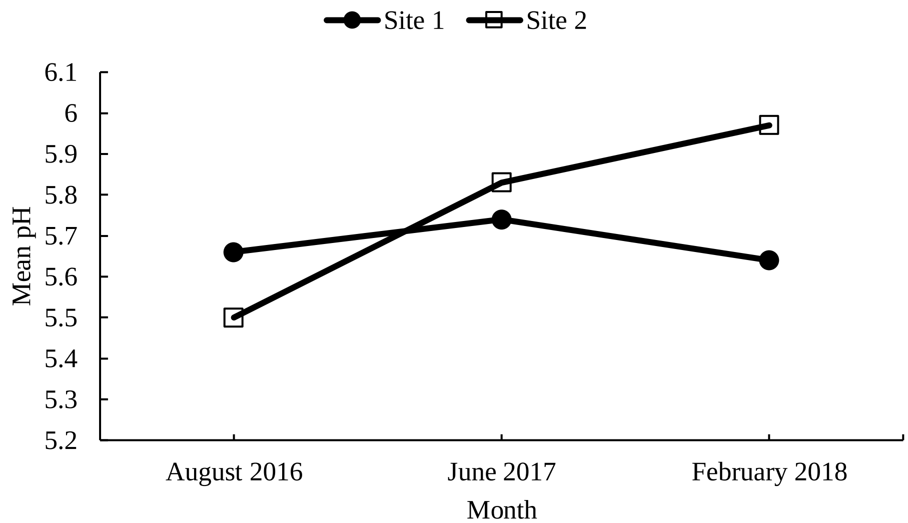

Figure 4.1- Mean soil pH averaged by location and by month for macadamia plots receiving six soil amendment treatments.

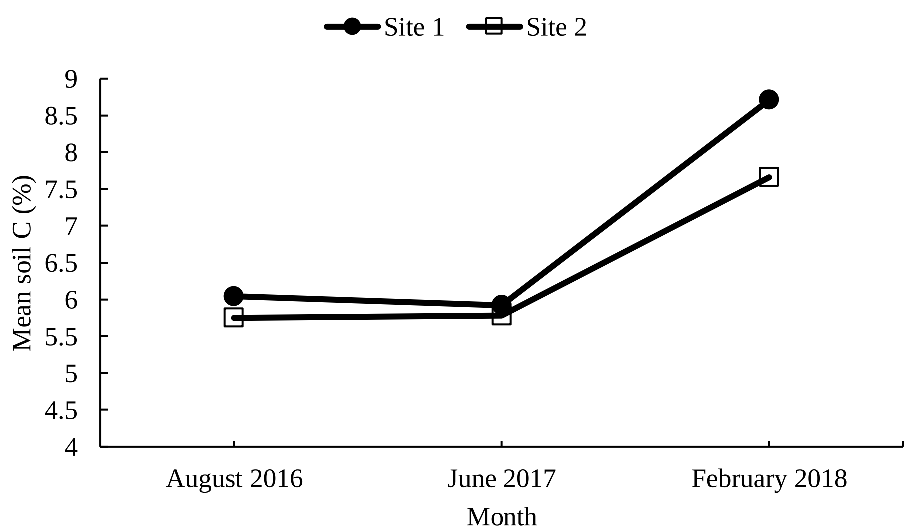

Figure 4.2- Mean soil C concentration averaged by location and by month for macadamia plots receiving six soil amendment treatments.

4.8 References

Allison, F.E. (1973). *Soil Organic Matter and its Role in Crop Production*. Amsterdam. Elsevier.

Bezborodov, G., Shadmanov, D., Mirhashimov, R., Yuldashev, T., Qureshi, A., Noble, A., Qadir, M. (2010). Mulching and water quality effects on soil salinity and sodicity dynamics and cotton productivity in Central Asia. *Agriculture, Ecosystem, and Environment.* 138, 95-102.

Bunemann, E., Schwenke, G., Van Zwieten, L. (2006). Impact of agricultural inputs on soil organisms- a review. *Australian Journal of Soil Research.* 44, 379-406.

Canbolat, M., Bilen, S., Cakmakci, R., Sahin, F. (2005). Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. *Biology and Fertility of Soils.* 42, 350-357.

Chan, K., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S. (2007). Agronomic values of greenwaste biochar as a soil amendment. *Australian Journal of Soil Research.* 45, 629-634.

Clough, T., Condron, L., Kammann, C., Muller, C. (2013). A review of biochar and soil nitrogen dynamics. *Agronomy.* 3, 275-293.

Dalby, T., Cox, J., Morris, S. (2010). Harvest equipment and soil erosion in a macadamia orchard. *19th World Congress of Soil Science, Soil Solutions for a Changing World*, 1-6 August 2010, Brisbane, Australia.

DeFrank, J., Foss, S. (1989). Yield and growth of macadamia trees in response to mulching with macadamia husks. *HortScience. 24*(2), 392.

Environmental Protection Agency. (2016). *Climate Change Indicators in the United States: Atmospheric Concentrations of Greenhouse Gases*. Accessed January 11, 2018. https://www.epa.gov/sites/production/files/2016-08/documents/print_ghg-concentrations-2016.pdf

Firth, D., Lobel, M., Johns, G. (1994). Effect of mulch, Ca, and Mg on growth, yield, and decline of Macadamia. *Journal of Tropical Agriculture*. *71*(3), 170-175.

Fox, R. (1973). Chemistry and management of soils dominated by amorphous colloids. *Soil Crop Science Society of Florida Proceedings.* 33, 112-119.

Giambelluca, T., Chen, Q. , Frazier, A., Price, J., Chen, Y., Chu, P., Eischeid, J. Delparte, D. (2013). Online Rainfall Atlas of Hawai‘i. *Bulletin of the American. Meteorological Society*. 94, 313-316.

Heisler, J., Glibert, P., Burkholder, J., Anderson, D., Cochlan, W., Dennison, W., Gobler, C., Dortch, Q., Heil, C., Humphries, E., Lewitus, A., Magnien, R., Marshall, H., Sellner, K., Stockwell, D., Stoecker, D., Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific concensus. *Harmful Algae*. *8*(1), 3-13.

Hueso-Gonzalez, P., Martinez-Murillo, J.F., Ruiz-Sinoga, J.D. (2014). The impact of organic amendments on forest soil properties under Mediterranean climatic conditions. *Land degradation & Development.* 25, 604-612.

Huett, D.O., B.J. Gogel, M.N. Meyers, C.A. McConchie, L.M. McFayden and S.C. Morris. (2001). Leaf nitrogen and phosphorus levels in macadamias in response to canopy position and light exposure, their potential as leaf-based shading indicators, and implications for diagnostic leaf sampling protocols. *Australian Journal of Agricultural Research.* 52, 513–522.

King, L. (1990). Sustainable soil fertility practices. In C.A. Francis et al. (ed.) *Sustainable agriculture in temperate zones.* New York, NY. Wiley-Interscience.

Magdoff, F., Weil, R. (2004). Significance of soil organic matter to soil quality and health. Magdoff, F., Weil, R. (Ed.'s), *Soil organic matter in sustainable agriculturen.* (pp. 1-43). CRC Press, Boca Raton, FL.

Major, J., Rondon, M., Molina, S., Lehmann, J. (2010). Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant and Soil. 333*(1), 117-128.

Nagao, M., Hirae, H. (1992). Macadamia: Cultivation and Physiology. *Critical Reviews in Plant Science*, *10*(5), 441-470.

Paul, E., Paustian, K., Elliot, E., Cole, C. (1996). *Soil Organic Matter in Agroecosystems*. Boca Raton, FL. CRC Press.

Porter, G., Yost, R., Nagao, M. (2005). the Application of Macadamia Nut Husk and Shell Mulch To Mature Macadamia Integrifolia To Improve Yields, Increase Nutrient Utilization, and Reduce Soil P Levels. *Western Nutrient Management Conference*. *6*, 226–233. Salt Lake City, UT.

Radovich, T. J. K., Cox, L. J., Hollyer, J. R. (2009). Overview of Organic Food Crop Systems in Hawaiʻi. College of Tropical Agriculture and Human Resources. SA-3.

Reid G . (2002). Soil and nutrient loss in macadamia lands: a pilot study. Horticulture Australia Report MC 98011.

Robertson, G. (1989). Nitrification and denitrification in humid tropical ecosystems: potential controls on nitrogen retention. In J. Proctor (Ed.) *Mineral nutrients in tropical forest and savanna ecosystems.* Cambridge, MA. Blackwell Scientific.

Sainju, U. (2017). Determination of nitrogen balance in agroecosystems. *MethodsX.* 4, 199-208.

Sanchez, J., Edson, C., Bird, G., Whalon, M., Wilson, T., Harwood, R., Kizilkaya, K., Nugent, J., Klein, W., Middleton, A., Loudon, T., Mutch, D., Scrimger, J. (2003). Orchard floor and nitrogen management influences soil and water quality and tart cherry yields. *Journal of the American Society of Horticultural Science 128*(2), 277-284.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda. gov/. Accessed [October/25/2016].

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Soil Series Classification Database. Available online at https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/class/?cid= nrcs142p2_053583. Accessed [March/8/2018].

Stephenson, R. and B.W. Cull. (1986). Flushing patterns of macadamia trees in south east Queensland. *Acta Horticulturae.* 175, 49– 53.

Stephenson, R., Gallagher, E., Doogan, V. (1997). Leaf nitrogen as a guide for fertilizing macadamia. *Australian Journal of Experimental Agriculture. 37*(5), 599-604.

Tamimi, Y., Silva, J., Yost, R., Hue, N. (1994). Adequate nutrient levels in soils and plants in Hawaii. CTAHR Fact Sheet No. 3. University of Hawai'i at Manoa, Honolulu, HI.

Theng, B. (1991). Soil science in the tropics-the next 75 years. *Soil Science*. 151, 76-90.

Youkhana, A., Idol, T. (2009). Tree pruning mulch increases soil C and N in a shaded coffee agroecosystem in Hawai'i. *Soil Biology & Biochemistry.* 41, 2527-2534.

Xue, N., Weiling, S., Huanchao, Z., Xiulian, Y., Lianggui, W. (2016). Effects of mulching on soil properties and growth of tea olive (*Osmanthus fragrant*). *PLoSONE*. *11*(8), 1-11.

CHAPTER 5

CONCLUSION

The SPAD-502 chlorophyll meter was evaluated for its use in determining leaf tissue N status in macadamia. The chlorophyll meter has the potential to be used in assessing leaf tissue N when not seeking absolute N concentrations. SPAD values were the most accurate at predicting leaf tissue N during the late winter to early spring, which is in line with current recommendations for tissue nutrient sampling. Modelling using higher order polynomial equations, adjusting for leaf moisture, using N mass per leaf area measurements, and taking more measurements on a single leaf may increase the performance of the chlorophyll meter. Macadamia cultivars differ in leaf nitrogen concentrations and may need distinct recommendations based on these differences depending on the sampling period.

Application of five soil amendments was evaluated from baseline soil sampling in 2016 through treatment application in 2017 and until the end of the harvest season in February 2018. Soil profiling had the greatest positive effect on yield. This management practice is a destructive method and may be detrimental to tree health if practiced annually in the same location. Determining the long-term effects of soil profiling on macadamia orchards would help inform growers of these risks. Soil profiling is also a relatively cheap practice compared to other soil amendments. Husk mulch in combination with biochar or the product EM1 have the potential to improve root growth. Husk mulch combined with EM1 in particular can increase proteoid root growth. Proteoid roots are implicated in improved nutrient and water uptake. The husk mulch in combination with EM1 and the soil profiling treatment were also responsible for significantly increasing SPAD values over a year time frame in the conventionally managed site. These two treatments have the potential to improve crop health variables that are of importance to growers. The cost of the EM1 treatment could deter growers from choosing this option. Costs can be reduced by increasing capacity of the spray equipment to reduce preparation time per acre. The benefit of these soil amendments was only assessed in economic terms based on yield increases for one year. A longer-term cost-benefit analysis would be necessary to define effects in a perennial cropping system. Potential benefits beyond yield increases are yield stability, soil quality, water quality, biodiversity, climate change mitigation, and worker health. Currently in Hawaiʻi, there are no processors that accept organic macadamia at a higher price per lb. than conventional macadamia, though this relationship is expected to change with the establishment of organic macadamia nut processing facilities.

The effects of the treatments on measured soil parameters were variable by location and resulted in less pronounced differences among treatments. While soil N did not vary significantly among treatments, the husk treatments in general resulted in higher increases in NO_3- and lower decreases in NH_4+ compared to the control. Effects on pH were greater in the conventional site compared to the organic site. The husk combination with EM1 had the greatest increase in soil C and the greatest decrease in pH in site 1. In site 2 the husk combination with biochar had the least increase in soil C and the greatest increase in soil pH. This may result in a combination of the biochar having a high baseline pH. It also demonstrates that higher soil C and implicitly higher SOM is a factor in increased pH buffering. Husk mulch has the potential to increase soil EC significantly, especially when in combination with EM1 and biochar. These increases over time may lead to detrimental EC values.

This research generates several new questions and areas to study further. Developing and improving the use of the SPAD chlorophyll meter for determining leaf N concentrations could focus on improving methods like taking multiple measurements on leaf subsamples, factoring in leaf moisture content, and using N mass per leaf area for N measurements. Additional macadamia cultivars and sampling periods could also be evaluated. Evaluating the use of soil amendments also elicited several new areas of potential research. Attention could be focused on evaluating the long-term effects of soil profiling as well as its effects on ABA, ethylene, and carbohydrate dynamics in macadamia and these effects on flowering and yield. An additional gap in knowledge are the effects and mechanisms in which *lactobacillus casei* may cause an increase in proteoid root growth in macadamia. Evaluating sugar signaling as a promoter of proteoid root growth in macadamia as well as the concentrations of sugars in mulches inoculated with *L. casei* would be one potential direction. A longer-term study of the studied soil amendments on tree and soil health deserves considerable attention. Macadamia is a relatively long-lived crop and maintaining soil health is an essential component to responsible land management in perennial systems. Macadamia growers should be considering the environmental and long-term production consequences that could occur from removing organic matter and creating a bare orchard floor. Using the results from this study can help to inform growers with interest in using some of these available soil amendment options.